The Role of HER1-HER4 and EGFRvIII in Hormone-Refractory Prostate Cancer
Joanne Edwards,1 Pamela Traynor,1 Alison F. Munro,1 Catherine F. Pirret,1 Barbara Dunne,2 and John M.S. Bartlett1

Abstract

Purpose: The role of the type I receptor tyrosine kinase (HER) family in progression of prostate cancer is controversial. Breast cancer studies show that these receptors should be investigated as a family. The current study investigates expression of HER1-HER4 and EGFRvIII in matched hormone-sensitive and hormone-refractory prostate tumors.

Experimental Design: Immunohistochemical analysis was used to investigate protein expression of HER1-HER4, EGFRvIII, and phosphorylated Akt (pAkt) in matched hormone-sensitive and hormone-refractory prostate tumors.

Results: Surprisingly, high HER2 membrane expression in hormone-sensitive tumors was associated with an increased time to biochemical relapse (P = 0.0003), and this translated into longer overall survival (P = 0.0021). Consistent with other studies, HER4 membrane expression in hormone-sensitive tumors was associated with longer time to biochemical relapse (P = 0.042), and EGFRvIII membrane expression was associated with shorter time to biochemical relapse (P = 0.015). An increase in pAkt expression was associated with reduced survival (P = 0.0098). Multivariate analysis showed that HER2 was an independent positive predictive marker of time to relapse in hormone-sensitive prostate tumors (P = 0.014). In contrast, high HER2 expression in hormone-refractory tumors was associated with decreased time to death from biochemical relapse (P = 0.039), and EGFRvIII nuclear expression was associated with decreased time to death from biochemical relapse and decreased overall survival (P = 0.02 and P = 0.005).

Conclusion: These results suggest that the HER family may have multiple roles in prostate cancer, and that expression of the proteins alone is insufficient to predict the biological response that they may elicit.

The HER family of receptors comprises four members: epidermal growth factor receptor (EGFR; erbB1/HER 1), HER2 (erbB2/neu), HER3 (erbB3), and HER4 (erbB4). In addition, variant receptors may be generated by alternative splicing (e.g., HER2/HER4) or mutations (particularly EGFR), and overexpression may occur as a result of gene amplification (especially HER2). The most frequently identified mutant form of EGFR is a constitutively active form called EGFR variant III (EGFRvIII; refs. 1, 2). The HER receptors play essential roles in the development and maintenance of mammary, cardiac, and neural tissues and have also been implicated in the development and progression of many cancers, including breast and prostate cancers (3, 4).

EGFR, HER3, and HER4 are activated via ligand binding, which results in the formation of homodimers or heterodimers with other family members (4). As HER2 ligands have not been identified, HER2 is believed to be activated by forming heterodimers with other family members (3, 5). Formation of HER receptor homodimers or heterodimers results in receptor activation via tyrosine kinase–mediated autophosphorylation, resulting in phosphorylation and activation of downstream pathways, such as the mitogen-activated protein kinase cascade and the phosphatidylinositol 3-kinase (PI3K)/Akt cascade (6). HER3 homodimers, however, are unable to activate downstream pathways as HER3 lacks intrinsic tyrosine kinase activity and is therefore dependent upon formation of heterodimers with other member of the HER family (3, 4, 6).

Aberrant activation of the HER family may occur via receptor overexpression, mutational activation, or increased growth factor concentrations. Increased activation of the HER family results in activation of mitogen-activated protein kinase and PI3K/Akt signaling cascades, culminating in increased cell proliferation and decreased cell death (3). It is therefore not surprising that modifications to the HER family are strongly associated with tumor formation and progression. Although the role of the HER family is well characterized in breast cancer,

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The role of the HER family is well characterized in breast cancer,
with drugs targeting both EGFR and HER2 shown to be effective in treating metastatic disease, the role of this family in the development and progression of prostate cancer remains controversial.

Data from breast and ovarian cancer suggest that because these receptors have similar function, they should be studied as a family. HER1-HER3 in breast cancer are associated with increased cellular proliferation, and HER4 seems to have a nonproliferative role (7, 8). Breast cancer patients with HER1-HER3-positive tumors have significantly poorer prognosis than those patients with HER-negative tumors or HER4-positive tumors (9). Therefore, in breast cancer, HER-targeted agents to EGFR and HER2 may be a more effective approach than a pan HER inhibitor.

Increased expressions of the EGFR, HER2, HER3, HER4, and EGFRvIII have all been described in prostate cancer (10–14). Data suggest that there is an increase in EGFR and HER2 expression at hormone relapse (10, 13, 15, 16). However, because EGFR and HER2 genes are not frequently amplified in prostate cancer (12, 13), this increase was not linked to gene amplification as in other cancers. Alternatively, the cell can regulate growth factor receptor expression via receptor degradation. Following ligand stimulation, the receptor is internalized where it is either degraded or recycled to the cell membrane to undergo further activation (3). Disruption of this degradation process may result in increased protein expression, independent of protein synthesis (3). This may be the mechanism employed in prostate cancer to increase HER2 expression. However, in the case of EGFR, loss of EGFR protein expression as the cancer progresses has been reported, and this is accompanied by an increase in expression of EGFRvIII (2), resulting in deregulated growth, independent of ligand activation. An increase in EGFRvIII expression may therefore be more common in prostate cancer than an increase in the wild-type EGFR.

HER3 and HER4 are expressed in 11% to 20% of breast cancers (9); however, few studies have investigated the role of HER3 and HER4 in prostate cancer. A recent study reported that levels of HER3 and HER4 do not change in the transition from hormone-sensitive to hormone-refractory prostate cells. However, high levels of HER4 in hormone-refractory tumors were linked to improved patient survival (10), consistent with observations made in breast cancer (7, 9).

To our knowledge, this is the first report that investigates expression levels of all four members of the HER family and EGFRvIII in matched hormone-sensitive and hormone-refractory tissue. This study aims to clarify the role of the HER family in the development of clinical HRPC. We hypothesize that HER1-HER3 cooperatively mediate hormone relapse and early death in prostate cancer patients. This study may provide evidence to support the use of a novel pan HER inhibitor in treatment of prostate cancer or may show that specific inhibitors of one family member would be a more effective approach.

Materials and Methods

Patient cohort. Seventy-four patients with matched hormone-sensitive and hormone-refractory tumor pairs were retrospectively selected for analysis. All tumors had patient identification removed, including block number and hospital number, and were coded to make the database anonymous. Ethical approval was obtained from the Multicentre Research Ethics Committee for Scotland (MREC/01/03/36) and Local Research and Ethical Committees. Patients were only selected for analysis if they initially responded to hormone treatment [response was defined by prostate-specific antigen (PSA) levels decreasing by at least 50% but subsequently relapsed (two consecutive increases in PSA of >10%) and had a pre-hormone and post-hormone relapse sample available for analysis. Therefore, tumors classed as hormone sensitive were from patients that were diagnosed with locally advanced (n = 56) or metastatic prostate cancer (n = 18) and received surgery, subsequently followed by hormone therapy in the form of androgen deprivation therapy (subcapsular bilateral orchidectomy and GnRH analogue) or maximum androgen blockade. To meet the inclusion criteria, a response to this therapy had to be observed; a response to therapy was defined by PSA levels decreasing by at least 50% and a nadir being reached. The hormone-sensitive tumor samples were obtained either from a TURP- or a TRUS-guided biopsy. In addition, patients were required to relapse with hormone-refractory prostate cancer to meet the inclusion criteria. A tumor was classified as being hormone refractory if the patients stopped responding to hormone therapy, this was defined as two consecutive increases in PSA concentration of >10%; the patients were also required to fail to respond to any alternative hormone therapies administered. However, following identification of such patients, refractory tumor samples were only available for analysis if additional surgery was required to treat clinical symptoms (bladder outflow obstruction). Therefore, hormone-refractory tumors samples were obtained only by TURP following failure of hormone therapy. In summary, all patients in this cohort initially respond to hormone therapy but subsequently relapsed with hormone-refractory disease; this provides matched hormone-sensitive and hormone-refractory tumors to track molecular changes associated with the transition from hormone-sensitive to hormone-refractory prostate cancer. Gene amplification status has previously been investigated by fluorescent in situ hybridization for 52 patients in this cohort for both EGFR and HER2 (13). No tumors were amplified for EGFR, and only 7% were amplified for the HER2 gene. These were low level amplifications and did not correlate with protein expression; it was, therefore, not deemed necessary to extend these studies to the full cohort (13).

Immunohistochemistry. All immunohistochemical analyses were done on 5-μm, archival formalin-fixed, paraffin-embedded prostate tumor sections. EGFR and HER2 immunohistochemistry was done as previously described (13). In brief, for EGFR immunohistochemistry, tissue was incubated with EGFR antibody (clone 31G7, Zymed, San Francisco, CA) at a 1:50 dilution for 1 hour at 25°C, for HER2 immunohistochemistry, the HercepTest (DakoCytomation, Glostrup, Denmark) and a Technem immunostainant (DakoCytomation) were used with strict adherence to kit protocol. Immunohistochemistry for EGFRvIII, HER3, HER4, and phosphorylated Akt at Ser643 (pAkt) were done as follows. Antigen retrieval for EGFRvIII was done using heat treatment under pressure in a Tris/EDTA Buffer (10 mmol/L Trizma base, 0.25 mmol/L EDTA) for 5 minutes. No antigen retrieval was required for HER3 and HER4, and antigen retrieval for pAkt was to heat in Tris/EDTA Buffer (10 mmol/L Trizma Base, 0.25 mmol/L EDTA) at 96°C for 20 minutes. HER3 and HER4 were blocked for endogenous biotin using an avidin/biotin blocking kit (Vector Labs, Peterborough, United Kingdom). Nonspecific background staining was blocked using either 5% horse serum in TBS for 1 hour (EGFRvIII, pAkt), 2.5% horse serum in TBS for 20 minutes (HER3), or serum-free blocking solution for 10 minutes (DakoCytomation; HER4). EGFRvIII (clone ZMD.82, Zymed), HER3 (clone H3:105.5, MS-303-PABX, Neomarkers, Fremont, CA), HER4 (clone HER1, MS-637-PO, Neo-markers), and pAkt (44-622G, Biosource, Camarillo, CA) antibodies were used at 1:500, 1:20, 1:50, and 1:100 dilutions, respectively. EGFRvIII, HER3, and HER4 were incubated for 2 hours at 25°C, and pAkt was incubated overnight at 4°C. Staining was developed using
either the LSAB plus kit (DakoCytomation) for EGFRvIII and HER4, the ImmPRESS anti-mouse immunoglobulin (peroxidase) kit (Vector Labs) for HER3 and EnVision kit (DakoCytomation) for pAkt. Chromagen was detected using 3,3’-diaminobenzidine (Vector Labs). A positive and negative control slide was included in each immunohistochemistry run; negative controls were incubated in an isotype-matched control antibody at a concentration of 1 mg/mL.

Tissue staining intensity was scored blind by two independent observers using a weighed histoscore method (17) also known as the isotype-matched control antibody at a concentration of 1 mg/mL. A positive and negative control slide was included in each immunohistochemistry run; negative controls were incubated in an isotype-matched control antibody at a concentration of 1 mg/mL.

Results

**Patient characteristics.** A total of 74 prostate cancer patients (diagnosed between 1984-2000) were included in this study with matched hormone-sensitive and hormone-refractory prostate tumors available for analysis (148 tumors in total). Patients in this cohort were diagnosed with locally advanced (n = 56) or metastatic prostate cancer (n = 18) and subsequently received surgery and androgen deprivation therapy (28 had subcapsular bilateral orchidectomy, 48 had GnRH analogue, two had both). Fifty-eight of the 74 patients also received antiandrogen therapy in the form of maximum androgen blockade, and this included all those who received GnRH analogues. At initial diagnosis, the median age was 70 years (range, 67-74 years), and 18% of patients had metastasis. The median time to biochemical relapse was 2.35 years (range, 1.48-4.40 years); the median PSA at relapse was 17 ng/mL (range, 6-41 ng/mL), and the percentage of patients with metastasis had increased to 35%. Sixty-seven patients died during follow-up, and the median survival for these patients was 4.39 (3.03-6.86). Seven patients were alive at last follow-up; the median time of follow-up for all 74 patients was 4.38 years (range, 2.75-6.93 years).

**Immunostaining.** Membrane protein expression and cytoplasmic protein expression was observed for all family members (although expression was very low for EGFR and HER2; Fig. 1; Table 1). Nuclear expression was observed for HER3, HER4, and EGFRvIII (Fig. 1; Table 1). To assess the level of agreement between observers, interclass correlation coefficients were calculated for each antibody at each location; all interclass correlation coefficients values in this study were >0.7 (which is classed as excellent; Table 1). The level of protein expression observed for EGFR and HER2 was lower than that observed for HER3, HER4, and EGFRvIII (Table 1). No overall significant increase was observed in median protein expression levels for any of the proteins investigated in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1). However, a significant decrease was observed for HER3 cytoplasmic expression in the transition from hormone-sensitive to hormone-refractory disease (Table 1).

### Table 1. Histoscore variation and comparison of staining intensity for hormone-sensitive and hormone-refractory tumors

<table>
<thead>
<tr>
<th></th>
<th>HSPC (IQR)</th>
<th>HRPC (IQR)</th>
<th>P</th>
<th>ICCC</th>
<th>Change</th>
<th>Fallers (%)</th>
<th>Risers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFRm</td>
<td>0 (0-9)</td>
<td>0 (0-18)</td>
<td>0.48</td>
<td>0.89</td>
<td>28</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>EGFRc</td>
<td>0 (0-0)</td>
<td>0 (0-0)</td>
<td>0.365</td>
<td>0.87</td>
<td>26</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HER2m</td>
<td>2.5 (0-30)</td>
<td>5 (0-22)</td>
<td>0.22</td>
<td>0.91</td>
<td>26</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>HER2c</td>
<td>0 (0-2.5)</td>
<td>0 (0-0)</td>
<td>0.985</td>
<td>0.85</td>
<td>24</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HER3m</td>
<td>85 (26-107)</td>
<td>75 (0-100)</td>
<td>0.06</td>
<td>0.95</td>
<td>48</td>
<td>32</td>
<td>14</td>
</tr>
<tr>
<td>HER3c</td>
<td>100 (78-136)</td>
<td>80 (60-100)</td>
<td>0.004</td>
<td>0.93</td>
<td>49</td>
<td>28</td>
<td>10</td>
</tr>
<tr>
<td>HER3n</td>
<td>20 (79-90)</td>
<td>55 (1-91)</td>
<td>0.349</td>
<td>0.95</td>
<td>34</td>
<td>24</td>
<td>42</td>
</tr>
<tr>
<td>HER4m</td>
<td>63 (5-100)</td>
<td>40 (75-200)</td>
<td>0.10</td>
<td>0.90</td>
<td>48</td>
<td>27</td>
<td>15</td>
</tr>
<tr>
<td>HER4c</td>
<td>75 (50-100)</td>
<td>50 (10-160)</td>
<td>0.094</td>
<td>0.90</td>
<td>47</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>HER4n</td>
<td>0 (0-20)</td>
<td>0 (0-18)</td>
<td>0.501</td>
<td>0.91</td>
<td>32</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>EGFRvIlm</td>
<td>200 (159-255)</td>
<td>200 (150-225)</td>
<td>0.09</td>
<td>0.82</td>
<td>75</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>EGFRvIlc</td>
<td>120 (100-150)</td>
<td>115 (100-150)</td>
<td>0.116</td>
<td>0.83</td>
<td>41</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>EGFRvIlv</td>
<td>130 (50-170)</td>
<td>70 (75-150)</td>
<td>0.133</td>
<td>0.98</td>
<td>31</td>
<td>20</td>
<td>31</td>
</tr>
</tbody>
</table>

**NOTE:** The median histoscore and interquartile range (IQR) for hormone-sensitive tumors and hormone-refractory tumors (HRPC) and the P of these values compared using a Wilcoxon sign rank test. The interclass correlation coefficient, which measures consistence between observers for each protein, is consistently >0.7, which is classed as excellent. The mean difference in observer scores plus 2 SDs is also shown as the number of histoscore units that is defined as a change in protein expression (change). The percentage of tumors that were defined as having a fall or rise in protein expression (calculated using the number of histoscore units that is defined as a change in expression) are also shown.

**Abbreviations:** IQR, interquartile range; HSPC, hormone-sensitive prostate cells; HRPC, hormone-refractory prostate cells; ICCC, interclass correlation coefficient.
to hormone-refractory disease \((P = 0.004; \text{Table 1})\). As described in Materials and Methods, a change in protein expression between matched hormone-sensitive and hormone-refractory tumor pairs is defined as the mean difference between the histoscores that each observer assigns for the protein expression plus 2 SDs. The number of histoscore units that represents a change in protein expression between tumor pairs for each protein is given in Table 1. Using this definition, it was noted that for each protein there were subgroups of patients whose tumors exhibited either a decrease or increase in protein expression (Table 1).

**Time to biochemical relapse and patient overall survival.** To determine if protein expression in the hormone-sensitive tumors was linked to time to biochemical relapse, Kaplan-Meier graphs of tumors expressing low levels of protein (<3rd quartile) versus high levels of protein (>3rd quartile) were plotted and compared using the log-rank test. The 3rd quartile was determined as the cutoff because the medians was not deemed as an appropriate cutoff for EGFR and HER2, as the medians were very low for both (0 and 2.5, respectively). However, when the histograms of score distribution for both proteins were plotted, the 3rd quartile seemed as the natural cutoff to use. Therefore, to keep the cutoffs consistent for all proteins, the 3rd quartile was employed as the cutoff for all proteins in this study. Using the 3rd quartile as the cutoff, neither EGFR nor HER3 expression, at any cellular location, was associated with time to biochemical relapse. However, those patients whose tumors expressed low levels of HER2 or HER4 in the membrane relapsed significantly earlier than those patients whose tumors expressed high levels of HER2 or HER4 in the membrane (Fig. 2A and B; \(P = 0.0001\) and \(P = 0.042\), respectively). In addition, this effect translated into a difference in overall survival. The median survival for patients whose tumors had low HER2 membrane expression is 4.2 years (range, 2.59-5.81 years) compared with 7.27 years (range, 4.48-10.06 years) for patients whose tumors expressed high levels (\(P = 0.0021\); HR, 0.31; 95% CI, 0.14-0.67). Similarly, the median survival for patients whose tumors expressed low levels of HER4 membrane expression is 4.92 years (range, 3.34-6.50 years) compared with 6.44 years (range, 4.96-7.93 years) for patients whose tumors had high HER4 expression (\(P = 0.027\); HR, 0.48; 95% CI, 0.24-0.991). HER2 and HER4 expression in the hormone-sensitive tumor at any other cellular location did not influence time to biochemical relapse or overall survival.

Conversely, patients with tumors that express high levels of membrane EGFRvIII relapse significantly earlier than those patients whose tumors expressed low levels of membrane EGFRvIII (Fig. 3; \(P = 0.015\)). Again, this effect translated into...
overall survival; those patients with tumors expressing high levels of membrane EGFRvIII have a median survival period of 3.92 years (range, 3.02-4.82 years) compared with those patients whose tumors expressed low levels of EGFRvIII in the membrane (P = 0.037; HR, 1.98; 95% CI, 1.07-3.68). Expression of EGFRvIII in the cytoplasm or nucleus was not related to time to biochemical relapse; however, those patients whose tumors expressed high levels of EGFRvIII in the cytoplasm had a significantly shorter survival period (4.36 years; range, 2.68-6.04 years) compared with those patients whose tumors expressed low levels of EGFRvIII in the cytoplasm (6.50 years; range, 5.48-7.52 years; P = 0.012; HR, 2.1; 95% CI, 1.15-3.91). Multivariate analysis showed that when expression of EGFR, HER2, HER3, HER4, and EGFRvIII is combined with Gleason and presence of metastases at diagnosis, only HER2 membrane expression was independently positively associated with time to relapse, suggesting that HER2 might be a positive independent predictive marker of time to relapse in hormone-sensitive tumors (P = 0.015). Gleason and presence of metastases at diagnosis were negatively associated with time to relapse and are known negative prognostic factors in prostate cancer (P = 0.020 and 0.031, respectively; Table 2). When the same variables were combined for overall survival, only Gleason and presence of metastases at diagnosis were independently associated with overall survival (P = 0.002 and P = 0.045, respectively; Table 2).

**Time to death from biochemical relapse and patient overall survival.** To determine if protein expression in the hormone-refractory tumors were linked to time to death from biochemical relapse, Kaplan-Meier graphs of tumors expressing low levels of protein and high levels of protein were plotted and compared using the log-rank test. EGFR, HER3, and HER4 were not significantly associated with time to death from biochemical relapse at any cellular location. However, those patients whose tumors expressed high levels of HER2 in the membrane of the hormone-refractory tumor died significantly earlier than those patients whose tumors expressed low levels of HER2 in the membrane (Fig. 4A; P = 0.0394; HR, 1.6; 95% CI, 0.80-3.19). This did not, however, translate into overall survival. In addition, those patients whose tumors expressed high levels of EGFRvIII in the nucleus of the hormone-refractory tumor died significantly earlier than those patients whose tumors expressed low levels of EGFRvIII in the nucleus (P = 0.02; HR, 1.4; 95% CI, 0.74-2.65). This effect also translated into overall survival (P = 0.0059; HR, 2.6; 95% CI, 1.29-5.49).

**Changes in protein expression with the development of hormone-refractory disease are associated with patient survival.** When time to death from biochemical relapse was investigated in relation to an increase in protein expression in the transition from hormone-sensitive to hormone-refractory disease, it was noted that only an increase in HER2 membrane expression was associated with decreased time to death from biochemical relapse (Fig. 4B; P = 0.012; HR, 2.62; 95% CI, 1.2-5.75). This effect did not translate into reduced overall patient survival and was not observed for any other protein at any other location.

**Correlation between protein expression of HER family members.** In the hormone-sensitive tumors, when protein expression levels (expressed as histoscore units) were correlated, EGFR protein expression weakly correlates with HER2 protein expression (membrane, correlation coefficient = 0.342 and P = 0.013; cytoplasmic, correlation coefficient = 0.397 and P = 0.004). EGFR membrane and cytoplasmic expression also

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**Table 2. Multivariate analysis**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Relative HR (95% CI)</th>
<th>P, time to relapse</th>
<th>Relative HR (95% CI)</th>
<th>P, overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>1.37 (0.32-5.88)</td>
<td>0.667</td>
<td>1.33 (0.18-10)</td>
<td>0.779</td>
</tr>
<tr>
<td>HER2</td>
<td>0.18 (0.04-0.76)</td>
<td>0.0155*</td>
<td>0.58 (0.12-2.7)</td>
<td>0.482</td>
</tr>
<tr>
<td>HER3</td>
<td>2.5 (0.64-9.20)</td>
<td>0.191</td>
<td>1.67 (0.34-6.17)</td>
<td>0.604</td>
</tr>
<tr>
<td>HER4</td>
<td>0.75 (0.28-2.11)</td>
<td>0.594</td>
<td>0.92 (0.32-2.68)</td>
<td>0.880</td>
</tr>
<tr>
<td>EGFRvIII</td>
<td>0.78 (0.25-2.50)</td>
<td>0.677</td>
<td>0.68 (0.19-2.38)</td>
<td>0.547</td>
</tr>
<tr>
<td>pAkt</td>
<td>1.81 (0.63-5.26)</td>
<td>0.269</td>
<td>1.17 (0.44-3.32)</td>
<td>0.741</td>
</tr>
<tr>
<td>Gleason</td>
<td>4.68 (1.28-12.21)</td>
<td>0.020*</td>
<td>13.2 (2.56-69.44)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Metastasis</td>
<td>4.41 (1.15-17.01)</td>
<td>0.031*</td>
<td>4.5 (0.99-19.01)</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

**NOTE:** P's gained for both time to relapse and overall survival when expression levels of EGFR, HER2, HER3 HER4, EGFRvIII, and pAkt were combined with Gleason sum and presence of metastases at diagnosis.

*Significant.
weakly negative correlates with EGFRvIII cytoplasmic expression (membrane, correlation coefficient = −0.285 and \( P = 0.031 \); cytoplasmic, correlation coefficient = −0.286 and \( P = 0.031 \)). In the hormone-refractory tumors, the positive correlation between EGFR and HER2 and the negative correlation between EGFR and EGFRvIII are lost. No other correlations were observed.

**Downstream signaling.** To establish if the downstream PI3K/Akt pathway is activated in this patient cohort, protein expression of activated pAkt at Ser473 was assessed in those patients with sufficient tumor material remaining for analysis (56 patients). Those patients with high pAkt expression in their primary tumors have a shorter time to death compared with those patients with low pAkt expression [Fig. 5A; median overall survival is 3.50 years (range, 2.52-4.48 years) versus 6.04 years (range, 4.29-7.79 years); \( P = 0.058 \)]. Although this did not reach significance, possibly due to the small patient number, an HR of 1.7 (95% CI, 0.97-2.29) was observed. In addition, an increase in pAkt expression in the transition from hormone-sensitive to hormone-refractory disease was observed in \( \sim 25\% \) of (13 of 56) patients, suggesting that this pathway is up-regulated in a subgroup of patients in the transition from hormone-sensitive to hormone-refractory disease. This increase in expression was shown to be associated with reduced patient survival. Patients with an increase in pAkt expression compared with patients with a decrease or no change in expression had a reduced survival period from biochemical relapse (median survival decrease, 1.58-0.74 years; \( P = 0.050 \); HR, 1.9; 95% CI, 0.98-3.68) and a reduced overall survival (Fig. 5B; median survival decrease, 5.82-3.36 years; \( P = 0.0098 \); HR, 2.3; 95% CI, 1.2-4.5).

**Discussion**

Although HER2 has considerable clinical importance in advanced breast cancer (7), its role in prostate cancer remains controversial. In the current study, we observed that those patients whose tumors had an increase in HER2 expression with the development of hormone-refractory disease survived for a significantly shorter period following biochemical relapse than those patients whose tumors had no change or a decrease in HER2 expression, consistent with our previous findings (13). We also observed that those patients whose hormone-refractory tumors expressed high levels of HER2 die significantly earlier than those with low levels of HER2 expression, consistent with Hernes et al., who also investigated HER2 expression in matched prostate cancer patient samples (10). These results fit with previous breast cancer studies that show that HER2 is linked to increased proliferation and decreased apoptosis, providing possible mechanisms for disease progression.
HER2 expression in prostate cancers is also linked with development of metastatic prostate cancer, and it is possible that patients with high HER2 in their hormone-refractory tumors represents a subgroup of patients at high risk of developing metastatic disease. This is also one of the roles shown for HER2 in breast cancer (20). HER2 has also been shown to be required for optimum androgen receptor activity; therefore, inhibition of HER2 in hormone-refractory tumors may be one method of inhibiting activation of the androgen receptor in the absence of androgens (21), as high HER2 expression in hormone-refractory prostate cancer tumors seems to be a negative predictive factor for response to therapy, as it is associated with a shorter survival period from biochemical relapse (21).

In contrast to our previous report, we found no link between patient outcome and an increase in EGFR expression in the transition from hormone-sensitive to hormone-refractory disease (13). A similar percentage of patients’ tumors were noted to have an increase in EGFR protein expression with the development of hormone-refractory disease (6 of 74, 8.1% in current study compared with 4 of 48, 8.3% in previous study); however, the follow-up is more mature in the current study. It is difficult to make any firm conclusions with so few numbers, but it seems that an increase in EGFR expression is not linked to decreased patient survival in prostate cancer or effects on a sufficiently small cohort (<10%) that is unlikely to be a valuable target in this context. However, high EGFRvIII expression in the hormone-sensitive tumors is associated with shorter time to biochemical relapse and also shorter overall survival. EGFRvIII has a constitutively active tyrosine kinase that signals most frequently via the PI3K cascade (22). Constitutive activation of the PI3K/Akt cascade in combination with loss of PTEN commonly observed in prostate cancer results in uncontrolled cell proliferation and reduced apoptosis (23). In the current study, an increase in expression of phosphorylated and hence activated of Akt is associated with a significant reduction in overall patient survival (P = 0.0098). Therefore, the current study suggests a role of EGFRvIII and PI3K/Akt cascade in progression of prostate cancer; this pathway is currently being investigated in this patient cohort.

Both high HER4 and HER2 protein expression in the hormone-sensitive prostate cell tumors were associated with increased time to biochemical relapse and increase overall survival. In the normal human prostate epithelium, HER4 expression is high and is reported to be coupled to differentiation, growth arrest, and tumor suppression (14, 24). It is, therefore, not surprising that when this receptor is expressed in tumor cells, the tumor seems less aggressive. When the androgen-insensitive prostate cancer cell lines DU145 and PC-3 are transfected with HER4, the cells undergo growth arrest (24); similar observations are made in breast cancer studies. HER4 transfection in breast cells results in reduced proliferation and increased apoptosis (25, 26). In breast cancer cells, the antiproliferative role of HER4 correlates with heregulin-induced HER4 tyrosine phosphorylation (27). Following degradation of HER4 in breast cancer cells by tumor necrosis factor α converting enzyme and presenilin-dependent γ secretase, the intercellular domain of HER4 is released (28). The intercellular domain of HER4 is then able to enter the cytoplasm and accumulates in the mitochondria, resulting in induction of apoptosis (28); HER4 may function similarly in prostate cancer.

HER2 may also signal for apoptosis following degradation in hormone-sensitive prostate cancer cells (29). However, it is more likely that HER2 itself is not responsible for the mechanism underlying the effect we are observing, as 86% of the group of hormone-sensitive tumors that express high HER2 levels express high levels of two or more family members, and 46% have high expression levels of three or more family members. In contrast, only 10% of low HER2 expressing hormone-sensitive tumors express high levels of two other family members. HER2 may act as a surrogate for a subset of tumors with markedly different biology and may not be solely related to the function of HER2 itself.

Different ligands and ligand concentrations can activate the HERs to signal via different pathways and induce different biological responses (27). Specifically, low concentrations of heregulin are mitogenic, whereas higher concentrations lead to differentiation and inhibition of cell growth (27). This may reflect the most likely explanation of why overexpression of HER2 (multiple HERs) is a positive predictive factor in hormone-sensitive tumors but a negative one in hormone-refractory tumors. Heresulin is present in hormone-naive prostate cancer specimens; however, in prostate cancer specimens from patients that have undergone androgen withdrawal, heresulin expression is no longer detectable (30). Heresulin treatment of androgen-sensitive LNCaP cells, which express HER2 and HER3, results in activation of the mHOG/p38 pathway, resulting in a significant reduction in cell proliferation and morphologic changes (cell clustering and increase cell to cell membrane contact), consistent with a more differentiated phenotype (14, 30). In contrast, treatment of the hormone-resistant cell line CWR-R1 with heresulin results in activation of HER2 and HER3, which signal via the mitogen-activated protein kinase and PI3K cascades, resulting in increase androgen receptor transactivation and increased proliferation (31).

In summary, this rigorously controlled study identified EGFRvIII and an increase in HER2 expression as prostate cancer risk factors. In contrast, high HER4 and HER2 (multiple HERs) expression in hormone-sensitive tumors seemed to have a protective role.

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The Role of HER1- HER4 and EGFRvIII in Hormone-Refractory Prostate Cancer

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