Her-2/neu Expression in Prostate Cancer: A Dynamic Process?

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ABSTRACT

The clinical effects of targeting Her-2/*neu* in prostate carcinoma are not known. This study explores the feasibility of molecular profiling to determine the correlation between Her-2/*neu* expression and hormonal sensitivity.

Patients with progressive androgen-independent prostate carcinoma were eligible to participate in the study. Her-2/*neu* expression was assessed on pretreatment tissue specimens and on bone marrow obtained in progressive androgen-independent disease. Her-2/neu expression was evaluated by immunohistochemistry and by fluorescence *in situ* hybridization in a consecutive series of 26 progressive androgen-independent prostate cancer patients.

Twenty four bone marrow biopsy specimens and 16 prostate biopsies from 26 patients were analyzed. These biopsies were categorized by androgen sensitivity at the time of the biopsy. In total, 90% of specimens from bone marrow were Her-2/*neu* positive, and 10% of the specimens were Her-2/*neu* negative. Of the prostate biopsies, all were from patients with androgen-dependent disease. Three of 13 androgen-dependent prostate biopsies (23%) overexpressed Her-2/*neu*. Of the 10 tumor samples analyzed by fluorescence *in situ* hybridization, genomic amplification of the Her-2/*neu* locus was not detected in any of the metastatic prostate tumors.

Her-2/*neu* expression varies with the clinical state of patients with prostate carcinoma: Accurate Her-2/*neu* profiling requires sampling metastatic tissue in patients with metastatic disease. Her-2/neu sampling from metastatic prostate carcinoma is not feasible until more reliable and practical methods can be developed.

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INTRODUCTION

In Europe, prostate cancer is the second most common cause of cancer in men; in 1999, an estimated 179,300 men were diagnosed with prostate cancer, and >37,000 died of the disease (1). The chance of a man developing invasive prostate cancer during his lifetime is 1 in 6 or 15.6%. The risk increases with age. At the age of 50 years, a man has a 42% chance of developing prostate cancer and a 2.9% chance of dying from the disease (2).

Hormonal therapy has been the mainstay of treatment for advanced prostate cancer for over five decades. Current clinical practices have been guided by the results of large-scale randomized trials that showed, when used alone, agents that block androgen production are comparable with respect to their activity, regardless of whether these effects are assessed by measurable tumor regression, normalization, or percentage decline of a tumor marker (prostate specific antigen), or subjective response (clinical benefit). Treatment of metastatic cancer with hormone therapy temporarily controls symptoms in 70–80% patients (3). However, for the majority of patients, the median duration of response is only 12 months. Once hormone-refractory disease is documented, treatment options are limited, and the prognosis is very poor. Most patients will die within 9–12 months (4).

Prostate carcinoma is a dynamic process in which different phases in the natural history of the disease may be characterized by unique biological mechanisms. The pathways that underlie tumor pathogenesis, growth, and resistance to treatment are increasingly understood in each clinical state, and novel biological agents that target these pathways are now available for clinical testing.

Profiling of Her-2/*neu* status has shown a range of findings in prostate carcinoma. Alterations in Her-2/*neu* expression as a tumor progresses from localized to metastatic disease and from androgen dependence to androgen independence has yet to be fully established; preliminary data suggest that Her-2/neu expression is not uniform across disease states (5, 6).

This study explored the feasibility of molecular profiling in prostate carcinoma to determine the correlation between hormonal status and Her-2/*neu* expression.

MATERIALS AND METHODS

This study began accruing patients on June 1st, 2001. The last patient was included on August 31, 2003. Informed consent was obtained from all patients.

Pathology Staining and Eligibility. To participate in this trial, patients were required to have progressive androgen-independent metastatic prostate carcinoma. Tissue from registered patients was tested for Her-2/*neu* expression. Overexpression was documented on the original diagnostic prostate biopsy or in the metastasic site previous to hormonal treatment and on bone marrow biopsy of metastatic disease after hormonal treatment.

Tissue Specimens and Decalcification. The bone marrow specimens used in this study had been fixed with 4%

buffered formalin and then decalcified using the chelating agent EDTA. A 10% EDTA solution was used in distilled water (pH 7.4) for a period of 2–3 weeks, depending on the degree of mineralization, with renewal of EDTA every week. Then the tissues were embedded in paraffin. Four-to-six μm sections were cut, mounted in sylanizated slides and then deparaffinized in xylene followed by immersion in 100% ethanol. We obtained serially sectioned hematoxilin-eosin, inmunohistochemistry, and fluorescence *in situ* hybridization (FISH) tissue sections from the same patient.

All tumors from patients were stained for Her-2/*neu* using the Herceptest immunohistochemistry kit (Dako, Carpinteria, CA). A single reference pathologist interpreted all biopsies. Tumors in which Her-2/*neu* was assessed at <2+ (moderate, circumferential membrane staining readily visible at ×10 magnification and observed in >10% of tumor cells) with the standard Dako kit were considered as not overexpressing the protein.

FISH. The probe from Vysis, Inc (Downers Grove, IL) was used. This probe consists of two different probes, one with the centromeric α-satellite probe specific for chromosome 17 (spectrum green) and a locus specific probe from Her-2/*neu* gene (spectrum orange). The Vysis probe was provided in denatured state, as single-strand DNA.

Deparaffinized tissue sections were treated with chlorhydric acid 0.2 N and then with sodium tiocianate to eliminate salt precipitates (Sigma-Aldrich Quimica SA, Madrid, Spain). Pretreated slides were incubated in a solution of proteinase K for 5 min at 37°C to digest cytoplasmatic membrane proteins. Then the slides were post-fixed in buffered formalin.

Pretreated tissue sections and probes were denatured at 78°C for 5 min and hybridized overnight at 37°C in Hybrite chamber (Vysis, Inc.). Washing was performed at 72°C in a solution of 2 × SSC/0.3% NP40 for 2 min. Tissue sections were counterstained with 10 μl of 4,6-diamino-2-phenilindole (DAPI counterstain; Vysis, Inc.).

Results were analyzed in a fluorescent Nikon (Eclipse 600) microscope using the Cytovision software. Tissue sections were scanned at low magnification (×100) with 4,6-diamino-2-phenilindole excitation at 367 nm to localize those areas that had histopathological characteristics established by examining a serially sectioned hematoxilin-eosin stained tissue section from the same patient.

Her-2/*neu* amplification was calculated dividing both the most frequent and largest values for Her-2/*neu* spots/nucleus by the most frequent and largest values of chromosome 17 centromere spots/nucleus. A minimum of 60 nucleuses were scored, and amplification was considered when the ratio was ≥2. In this study, a minimum of 60 nuclei per case were studied by two different observers.

Table 1 Characteristics of the 15 patients analyzed

Characteristic	Total (n = 15 patients)
Median age (range)	70 years (55–86 year)
Median Karnofsky performance status (range)	90% (70–90%)
Baseline median biochemical parameters (range)	
Hemoglobin (g/dl)	11.2 (7.8–13.3)
LDH[a] (unit/liter)	352 (240–638)
PSA (ng/ml)	71.1 (0.15–653)
Previous hormonal therapy lines (range)	1 (1–2)

[a] LDH, lactate dehydrogenase; PSA, prostate specific antigen.

Table 2 Her-2/*neu* testing by immunohistochemistry in androgen-sensitive (AS)/primary prostate cancer and in androgen-independent (AI)/metastasic lesion and by fluorescence *in situ* hybridization (FISH) in AI/metastasic lesion in matched pair of patients.

Case	AS/primary prostate tumor	AR/metastasic lesion	Assay for Her-2/*neu* gene amplification by FISH
1	1+	2+	Negative
2	1+	1+	Negative
3	2+	2+	Negative
4	1+	2+	Negative
5	1+	3+	Negative
6	2+	2+	Negative
7	1+	2+	Negative
8	1+	2+	Negative
Total	2/8	7/8	0/8

NOTE. n = 8

RESULTS

Patients. The figures for screened patients are described in Table 1. Twenty six patients were screened. The median age was 70 years (range, 47–84 years). Of the screened patients, it was not possible to acquire primary tumor-containing tissue from 16 patients, because bone marrow biopsy failed to produce tumor (14 patients) or technical implications prevented to perform the biopsy in two patients. Also, in 13 patients archival tissue could be obtained. In total ten bone marrow samples were obtained for Her-2/*neu* testing.

There were eight matched pairs of patients, and the data are summarized in Table 2.

Pathology Studies. Twenty four bone marrow biopsy specimens and sixteen prostate biopsies from the 26 patients were analyzed. These biopsies were categorized by androgen sensitivity at the time of the biopsy. In total, 90% of specimens from bone marrow were Her-2/*neu* positive, and 10% of specimens were Her-2/*neu* negative. Of the prostate biopsies, all were from patients with androgen-dependent (AD) disease. Three of 13 AD prostate biopsies (23%) overexpressed Her-2/*neu*. Two of the prostate biopsies from the same patient varied in Her-2/*neu* expression.

Overall, there were eight matched pairs in which both the AD primary prostate specimen, and the androgen-independent (AI) metastatic tissue were available for Her-2/*neu* testing (Table 2). In seven samples, the AI metastases were Her-2/*neu* positive, and the corresponding AD prostate samples were Her-2/*neu* negative. One matched pair was concordantly Her-2/*neu* negative.

We also analyzed all subsets of AI tumors for evidence of genomic amplification. Of the 10 tumor samples analyzed by FISH, genomic amplification of the Her-2/*neu* locus was not detected in any of the metastatic prostate tumors. The breast carcinoma used as the positive control showed amplification of the Her-2/*neu* locus (FISH score > 10), whereas the one used as the negative control showed no amplification (Table 3).

Table 3 Her-2/*neu* testing by immunohistochemistry in androgen-sensitive, (AS)/primary prostate cancer and in androgen-independent, (AI)/metastasic lesion and by fluorescence *in situ* hybridization (FISH) in AI/metastasic lesion of 15 patients

Case	AS/primary prostate tumor	AI/metastasic lesion	Assay for Her-2/*neu* gene amplification by FISH
Maches pairs			
1	1+	2+	Negative
2	1+	1+	Negative
3	2+	2+	Negative
4	1+	2+	Negative
5	1+	3+	Negative
6	2+	2+	Negative
7	1+	2+	Negative
8	1+	2+	Negative
AI specimens			
9		2+	Negative
10		2+	Negative
AS specimens			
11	1+	Unknown	
12	1+	Unknown	
13	1+	Unknown	
14	1+	Unknown	
15	2+	Unknown	
Total	3/13	9/10	10/10

DISCUSSION

This trial was designed to test the hypothesis that there might be a correlation between hormonal insensitivity and Her-2/*neu* gene expression and to quantitatively determine this correlation. Given the plethora of studies using biological agents, we think that screening for the targeted pathway must occur on tissue that represents the disease at the time of treatment, not at diagnosis.

Our pathology results prospectively support what retrospective analyses have suggested previously: that Her-2/*neu* expression in prostate carcinoma varies with clinical state. We screened 26 patients but identified only 10 eligible patients with bone infiltration. Her-2/*neu* overexpression was found in 9 of the 10 AI metastases compared with 3 of the 13 prostate cancer tissue samples. Recently, a study on mice with detailed histological and immunohistochemical analyses revealed that the expression of other markers such as platelet-derived growth factor and platelet-derived growth factor receptor were restricted to PC-3MM2 cells growing adjacent to the mouse bone. Also, the authors demonstrated that platelet-derived growth factor receptor was expressed on endothelial cells within tumor lesions in the bone but not in the muscle (7). In this study, it was found that in five of eight matched pairs, the AD prostate biopsy did not overexpress Her-2/*neu*, but the AI metastatic sample did, providing evidence that variation in Her-2/*neu* expression can be documented even within individual patients as their disease progresses. A heterogeneous disease such as prostate cancer requires multimodal therapy and the translation of these findings to the clinical reality.

These results confirm the findings of Signoretti *et al.,* (8) who found that Her-2/*neu* is overexpressed in 25% of untreated prostate cancer tissue samples, in 59% of specimens treated with hormones before surgery and in 78% of patients with AI disease. These data suggest that accurate Her-2/*neu* profiling of patients with metastatic disease requires testing tissue at all points in the natural history of the disease, with a particular focus on acquiring metastatic tissue.

The mechanisms responsible for the development of androgen independence are not yet completely understood. However, in the past few years, several experimental studies have proposed that AR mutations, AR gene amplification, and alterations in growth factor-activated pathways can modulate AR signaling and, therefore, may play a key role in the progression to androgen-independent, hormone-refractory prostate cancer (9–11). The majority of androgen-independent prostate cancers express AR, suggesting that the AR signaling pathway is activated also in absence of androgen (12). In this respect, a functional cross-talk between growth factors and growth factor receptors of the EGFR family and AR-activated pathways has been shown in preclinical models (13–15). Craft *et al.* (16) have shown that androgen-independent sublines of LAPC-4 prostate cancer cells express high levels of c-erbB-2 and that overexpression of c-erbB-2 in parental LAPC-4 cells causes androgen-independent tumor growth. Furthermore, c-erbB-2 activates the AR pathway in the absence of ligand and synergizes with low levels of androgen to increase AR signaling in this prostate cancer cell model. Collectively, these results strongly support a role for Her-2/*neu* expression in the development of prostate cancer and, more specifically, in the progression to an androgen-independent, hormone-refractory clinical behavior.

Acquiring metastatic tissue from patients with prostate carcinoma is problematic. In this study, blind bone marrow biopsies yielded tumor in only 41% of patients. Another option would be to screen only patients with soft tissue disease, although such patients are in the minority, and tumors in lymph nodes may differ biologically from those in bone. One proposed solution to the difficulties of obtaining metastatic tissue for screening is to use shed serum Her-2/*neu* circulating epithelial cells (17).

Our results and others have found no gene amplification, even in specimens with protein overexpression, suggesting that FISH is a limited tool for screening (18). Recently, a very elegant study from Calvo *et al.* (19) reported the lack of Her-2/Neu overexpression in AI prostate cancer found by immunohistochemistry, mRNA tumor expression, or gene amplification. An important issue that needs additional investigation is whether c-erbB-2 protein overexpression is accompanied by the activation of growth factor receptor-induced intracellular downstream signaling. In this respect, it will be interesting to evaluate the activation of the mitogen-activated protein kinase and phosphatidylinositol 3′-kinase-AKT pathways.

This study underscores the difficulties of conducting clinical trials of biological agents in patients with prostate carcinoma. Such trials face the 4-fold challenge of developing validated assays of active pathways, acquiring tissue, establishing that a pathway is clinically relevant at the time of treatment, and determining meaningful end points. Future trials on biological agents in patients with metastatic prostate carcinoma will require new techniques for assessing biological pathways in metastatic tissue, and the assays used to test for these pathways require further validation. Prostate carcinoma is a dynamic

process in which different phases in the natural history of the disease may be characterized by unique biological mechanisms.

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