

·Original Article·

# Human epidermal growth factor receptor type 2 protein expression in Chinese metastatic prostate cancer patients correlates with cancer specific survival and increases after exposure to hormonal therapy

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## Abstract

**Aim:** To investigate human epidermal growth factor receptor type 2 (HER2) protein expression and gene amplification in Chinese metastatic prostate cancer patients and their potential value as prognostic factors. **Methods:** Immunohistochemistry (IHC) was performed to investigate HER2 protein expression in prostate biopsy specimens from 104 Chinese metastatic prostate cancer patients. After 3–11 months of hormonal therapy, 12 patients underwent transurethral resection of the prostate (TURP). HER2 protein expression of TURP specimens was compared with that of the original biopsy specimens. Of these, 10 biopsy and 4 TURP specimens with HER2 IHC staining scores  $\geq 2+$  were investigated for HER2 gene amplification status by fluorescent *in situ* hybridization (FISH). **Results:** Of the 104 prostate biopsy specimens, HER2 protein expression was 0, 1+, 2+ and 3+ in 49 (47.1%), 45 (43.3%), 8 (7.7%) and 2 (1.9%) cases, respectively. There was a significant association between HER2 expression and Gleason score ( $P = 0.026$ ). HER2 protein expression of prostate cancer tissues increased in 33.3% of patients after hormonal therapy. None of the 14 specimens with HER2 IHC scores  $\geq 2+$  showed HER2 gene amplification. Patients with HER2 scores  $\geq 2+$  had a significantly higher chance of dying from prostate cancer than those with HER2 scores of 0 ( $P = 0.004$ ) and 1+ ( $P = 0.034$ ). Multivariate Cox regression analysis showed that HER2 protein expression intensity was an independent predictor of cancer-related death ( $P = 0.039$ ). **Conclusion:** An HER2 IHC score  $\geq 2+$  should be defined as HER2 protein overexpression in prostate cancer. Overexpression of HER2 protein in cancer tissue might suggest an increased risk of dying from prostate cancer. HER2 protein expression increases in some individual patients after hormonal therapy. (*Asian J Androl* 2008 Sep; 10: 701–709)

**Keywords:** prostatic neoplasms; human epidermal growth factor receptor type 2; immunohistochemistry; gene amplification; prostate cancer; prognosis

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## 1 Introduction

Prostate cancer is the most common cancer and the second-leading cause of cancer related death among men in the USA [1]. The incidence rate of prostate cancer

varies widely among countries and racial groups. In 2002, the prostate cancer incidence rate was 15.6/100 000 in the city of Shanghai in China [2]. Although the rate is not currently very high in China, it has increased dramatically over the past 2 decades as a result of economic development and changing lifestyles [3]. Furthermore, most newly diagnosed Chinese prostate cancer patients are symptomatic and have metastatic diseases, because screening for prostate cancer using prostate specific antigen (PSA) and digital rectal examination is not a routine practice in China [3].

The primary treatment for metastatic prostate cancer is androgen deprivation therapy (ADT). Nevertheless, resistance to ADT is a major problem worldwide in prostate cancer treatment [4]. Currently, the mechanisms responsible for androgen independence are not clear. Knowledge of molecular changes caused by ADT might lead to better understanding of the events resulting in androgen independence [4].

Human epidermal growth factor receptor type 2 (HER2, also referred to as HER2/neu or ErbB-2) is a 185-kDa transmembrane tyrosine kinase receptor and it belongs to the epidermal growth factor receptor family [5]. The HER2 gene is located at chromosomal 17q21 and HER2 signaling promotes cell proliferation through the RAS–MAPK pathway and inhibits cell death through the phosphatidylinositol 3'-kinase–AKT–mammalian target of rapamycin pathway [5]. Preclinical studies suggest that HER2 expression plays a role in prostate cancer progression. Using a prostate cancer xenograft model, Craft *et al.* [6] demonstrated that androgen independent xenografts of LAPC-4 cells have higher HER2 protein levels than androgen dependent parental LAPC-4 cells. Forced overexpression of HER2 in androgen dependent LNCaP cells induces androgen independent growth *in vitro* and accelerates the progression to androgen independence in castrated animals [6].

The HER2 protein expression and gene amplification have been investigated in human prostate cancer patients. However, the results are controversial in these studies and there is no study including Chinese patients. Therefore, we performed this study on Chinese metastatic prostate cancer patients to explore the relation between HER2 status and patient outcome.

## 2 Materials and methods

### 2.1 Patients and tissue samples

The present study included materials from 104 Chinese patients who were diagnosed with metastatic prostate cancer and treated between 2000 and 2006 at Shanghai Cancer Hospital, Fudan University (Shanghai, China). This study was approved by the Institutional Review Board of the hospital. Written informed consent was obtained from each patient before any specific investigations were undertaken.

The diagnosis of prostate cancer was established by prostate biopsy in each patient. Clinical data including age, PSA level and tumor node metastasis (TNM) staging were obtained from medical records. Metastatic diseases were confirmed using imaging studies, including bone scanning, computed tomography and magnetic resonance imaging. The median age of patients was 70 years (range: 43–90 years) and the median serum PSA level of patients was 128.7 ng/mL (range: 10.5–6006.2) at the time of diagnosis. All patients were treated by maximal androgen blockade (MAB), with 44 (42.3%) patients receiving luteinizing hormone-releasing hormone agonists with antiandrogen agents (flutamide) and 60 (57.7%) patients receiving bilateral orchiectomy with antiandrogen agents (flutamide). Twelve (11.5%) patients in this study underwent transurethral resection of the prostate (TURP) to relieve urinary obstruction after at least 3 months of MAB therapy. All patients included in this study were routinely followed up according to European Association of Urology (EAU) guidelines. The median follow-up period was 34.5 months (range 16–94 months) for the censored patients and 18.5 months (range: 7–57 months) for patients who died of prostate cancer. During follow-up, 69.2% (72/104) of patients experienced PSA progression (PSA recurrence). Although secondary hormonal therapy (e.g. bicalutamide and ketoconazole) and chemotherapy (docetaxel + prednisone, mitoxantrone + prednisone) was administered for those with androgen independent prostate cancer and hormone refractory prostate cancer, respectively, 47 (45.2%) patients died at the end of this study.

Specimens from the original diagnostic prostate biopsy and the corresponding TURP were obtained. First, the hematoxylin and eosin stained histological sections of each specimen were reviewed and tumor grade was determined according to the Gleason system. Then, one representative tissue block with a high carcinoma content was chosen for further studies.

### 2.2 Immunohistochemical (IHC) analysis

The IHC was performed by Envision system. The 4- $\mu$ m-thick sections from the formalin fixed, paraffin embedded representative prostate cancer tissue blocks were mounted on aminopropyltriethoxy silane coated slides. The slides were heated to 60°C for 2 h and deparaffinized with xylene and rehydrated through 100% to 85% ethanol. Endogenous peroxidase was quenched in 3% hydrogen peroxide. Antigen retrieval was performed by soaking the slides in 0.01 mol/L (pH 6.0) citrated buffer and heating in a 99°C water bath for 30 min. The slides were cooled for 20 min at room temperature and rinsed in Tris buffered saline (TBS). The slides were incubated overnight at room temperature with polyclonal rabbit anti-human HER2 antibody (Code No. A0485, DakoCytomation, Glostrup, Denmark) at 1:300 dilution in TBS. After three sequential washes in TBS, the slides were incubated with secondary antibody for 30 min. Then, the slides were rinsed and incubated with 3-3'-diaminobenzidine (DAB) for 15 min. Hematoxylin was used for counterstaining. Paraffin sections from a breast cancer tissue block, which were known to be positive for HER2, served as positive controls. Omission of the primary antibody with TBS served as negative controls.

All slides were interpreted by two experienced pathologists in an open discussion. The pathologists were blinded to all clinical data. HER2 staining was scored from 0 to 3+ in accordance with the previous guidelines for scoring the HercepTest [7–9]. A score of 0 was assigned to no staining or faint membrane staining in < 10% of infiltrating tumor cells. A score of 1+ indicated a faint or barely perceptible membrane staining in > 10% of infiltrating tumor cells. A score of 2+ indicated a weak to moderate complete membrane staining in > 10% of infiltrating tumor cells. A score of 3+ meant strong complete membrane staining in > 10% of infiltrating tumor cells.

### 2.3 Fluorescence in situ hybridization (FISH) analysis

Sections with HER2 staining scores of 2+ or 3+ were further investigated for the HER2/neu gene amplification status using the PathVysion HER2/neu FISH kit (Vysis, Downers Grove, IL, USA). Sections were deparaffinized in xylene thrice for 10 min, immersed thrice with 100% ethanol, treated with pretreatment solution at 80°C for 30 min, digested with protease solution, and subsequently denatured. The Vysis probe consists of two different probes, a chromosome enumeration probe specific for chromosome 17 (CEP) (spectrum green) and a

locus specific identifier for HER2/neu gene (spectrum orange). Tissue sections and probes were denatured at 78°C for 5 min and hybridized overnight at 37°C. Tissue sections were counterstained with 10  $\mu$ L of 4,6-diamino-2-phenylindole.

Results were analyzed in fluorescent microscope (Olympus BX51, Tokyo, Japan) using the Cytovision software (Lecia). For each section, at least 60 non-overlapping nuclei were counted in contiguous 1 000  $\times$  microscopic fields. The signals per nucleus for the HER2 gene and CEP17 were counted on a cell-to-cell basis. Nuclei with either no signal for HER2 or CEP17, or only one color, were not counted [7, 10]. FISH was considered successful in each case in this study, because more than 90% of cells per case had both HER2 and CEP17 signals. An individual case was defined as having HER2 gene amplification when the ratio between the total number of HER2 and CEP17 signals was 2.0 or more [7, 10].

### 2.4 Statistical analysis

SPSS 13.0 (SPSS, Chicago, IL, USA) and SAS 8.0 (North California, USA) were used for the statistical analysis. The Cochran–Armitage trend test was used to test trends in patient age, tumor Gleason score and sub-stage of metastatic prostate cancer across HER2 protein expression intensity. The nonparametric Kruskal–Wallis *H*-test was used to analyze the difference in serum PSA levels in patients with different HER2 protein expression. The Mann–Whitney *U*-test was used to compare the HER2 protein expression in biopsy and TURP specimens from 12 matched pairs. The survival period was analyzed using the Kaplan–Meier method and a survival curve comparison between different groups was performed using the log rank test. Univariate and multivariate analysis of risk factors predicting prostate cancer specific survival was performed using the Cox proportional hazards regression model. All statistical tests were two-sided and *P* < 0.05 was considered statistically significant.

## 3 Results

### 3.1 HER2 protein expression by IHC analysis

In the whole study group, the HER2 staining score was 0, 1+, 2+ and 3+ in 49 (47.1%), 45 (43.3%), 8 (7.7%) and 2 (1.9%) cases, respectively (Figure 1). The Cochran–Armitage trend test determined that there was a statistically significant trend in tumor Gleason score across HER2 staining intensity. The tumor with a Gleason score

of 8–10 was associated with higher HER2 protein expression ( $P = 0.026$ , Table 1). There was no significant association between HER2 staining intensity and patient age, serum PSA level or substage of metastatic prostate cancer (Table 1). A total of 12 patients in this study underwent TURP after 3–11 months of MAB therapy and HER2 protein expression of the original diagnostic prostate biopsy specimen was compared with the subsequent TURP specimen in these patients (Table 2). In 4 (33.3%) patients, the HER2 protein expression increased intensity after MAB therapy (Table 2 and Figure 2). The Mann–Whitney U-test did not indicate that there was a statistically significant increase in the HER2 protein expression intensity in our patients after a period of MAB therapy ( $P = 0.059$ , Table 2).

### 3.2 HER2/neu gene amplification by FISH

We further investigated 10 biopsy specimens and 4

TURP specimens with HER2 staining scores of 2+ or 3+ for HER2/neu gene amplification status. None of the 14 specimens showed HER2 gene amplification by FISH.

### 3.3 Association of HER2 protein expression with cancer specific survival

At the end of this study, 47 (45.2%) patients died of prostate cancer. The 5-year cancer specific survival rate was 50.2%, 29.2% and 15.0% in patients with HER2 staining scores of 0, 1+ and  $\geq 2+$ , respectively (Figure 3). Patients with HER2 score  $\geq 2+$  had a significantly higher chance of dying from prostate cancer than those with HER2 scores of 0 ( $P = 0.004$ ) and 1+ ( $P = 0.034$ ). However, there was no significant difference in the cancer specific survival rate between the HER2 (score 0) group and the HER2 (score 1+) group ( $P = 0.339$ ). To further investigate the significant prognostic factors associated with prostate cancer specific survival the

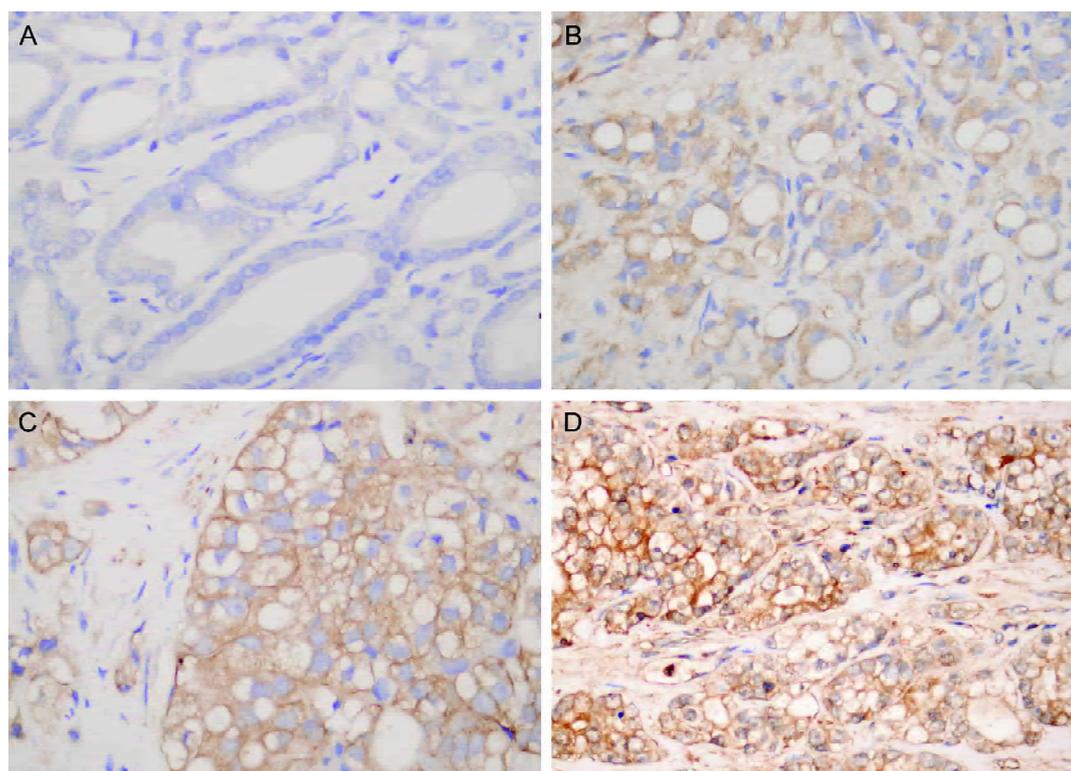


Figure 1. Immunohistochemical (IHC) analysis of human epidermal growth factor receptor type 2 (HER2) protein expression in original diagnostic prostate biopsy specimens. (A): Gleason grade 3 tumor shows no membrane staining in tumor cells (HER2 score 0). (B): Gleason grade 4 tumor shows faint or barely perceptible membrane staining in  $> 10\%$  of tumor cells (HER2 score 1+). (C): Gleason grade 5 tumor shows weak to moderate complete membrane staining in  $> 10\%$  of tumor cells (HER2 score 2+). (D): Gleason grade 5 tumor shows strong complete membrane staining in  $> 10\%$  of tumor cells (HER2 score 3+). Reduced from  $\times 400$ .

Table 1. Comparison of human epidermal growth factor receptor type 2 (HER2) protein expression according to different patient characteristic. \*Cochran-Armitage trend test. †Kruskal-Wallis *H*-test. §Prostate cancer. PSA, prostate specific antigen.

	HER2 protein expression (%)				Total	P-value
	0	1+	2+	3+		
Number of patients (%)	49 (47.1)	45 (43.3)	8 (7.7)	2 (1.9)	104	
Age range (%)						
< 70 years	21 (44.7)	19 (40.4)	5 (10.6)	2 (4.3)	47	0.368*
≥ 70 years	28 (49.1)	26 (45.6)	3 (5.3)	0 (0)	57	
Gleason score (%)						
6–7	28 (57.1)	19 (38.8)	2 (4.1)	0 (0)	49	0.026*
8–10	21 (38.2)	26 (47.3)	6 (10.9)	2 (3.6)	55	
Serum PSA level (ng/mL)						
Range	10.8–3808.5	13.5–6006.2	101.2–713.4	140.1–261.4		0.661†
Median	107.0	130.3	174.2	200.8		
Substage of metastatic PCa (%)§						
M1a (involvement of non-regional lymph nodes)	4 (50.0)	4 (50.0)	0 (0)	0 (0)	8	0.178*
M1b (involvement of bones)	42 (48.3)	38 (43.7)	6 (6.9)	1 (1.1)	87	
M1c (involvement of other distant sites)	3 (33.3)	3 (33.3)	2 (22.2)	1 (11.1)	9	

Table 2. Comparison of human epidermal growth factor receptor type 2 (HER2) protein expression in 12 matched pairs. \*Mann-Whitney *U*-test. MAB, maximal androgen blockade; TURP, transurethral resection of prostate.

Case	Age (years)	Time of MAB (months)	HER2 protein expression		P-value
			Biopsy specimen	TURP specimen	
1	56	11	0	0	0.059*
2	72	3	0	0	
3	57	6	0	0	
4	71	7	0	0	
5	67	5	0	2+	
6	59	3	0	2+	
7	74	5	0	2+	
8	48	3	1+	1+	
9	63	3	1+	1+	
10	70	8	1+	1+	
11	59	5	1+	1+	
12	63	4	1+	2+	

univariate and multivariate risk factor analyses were performed using the Cox proportional hazards regression model (Table 3). Tumor Gleason score, substage of metastatic prostate cancer and HER2 protein expression were significant univariate predictors of death from prostate cancer (Table 3). These results corresponded to the Kaplan–Meier survival analysis curves (Figures 3–5). However, multivariate analysis of these factors showed that HER2 protein expression and substage of metastatic

prostate cancer were independent predictors of cancer-related death (Table 3). They remained significant variables in the forward and backward stepwise calculation models.

#### 4 Discussion

ADT has been the mainstay of treatment for metastatic and advanced prostate cancer for over 5 decades.

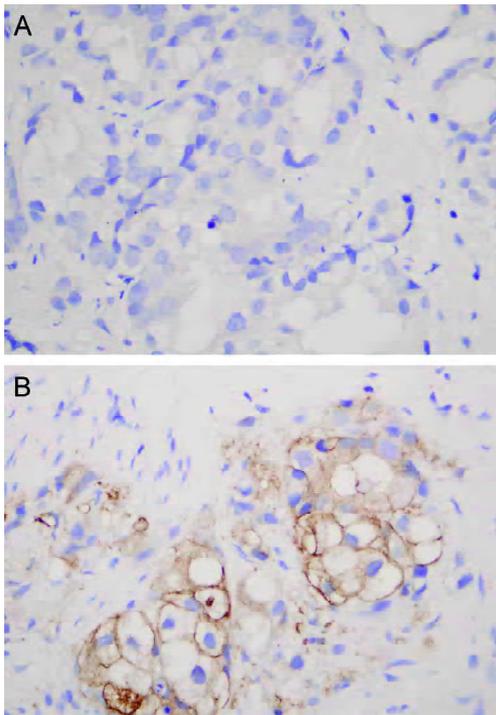


Figure 2. Comparison of human epidermal growth factor receptor type 2 (HER2) protein expression in original diagnostic prostate biopsy specimen and subsequent transurethral resection of prostate (TURP) specimen from the same patient. (A): Biopsy specimen shows HER2 score 0. (B): TURP specimen shows HER2 score 2+ and cancer cells exhibit xanthomatous cytoplasm within the fibrotic background, which indicates a response to hormonal therapy. Reduced from  $\times 400$ .

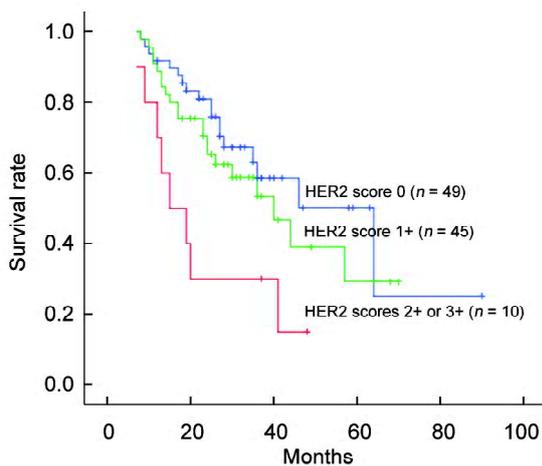


Figure 3. Prostate cancer specific survival curve for human epidermal growth factor receptor type 2 (HER2) immuno-histochemistry (IHC) scores of 0, 1+ and  $\geq 2+$  groups. HER2 score 0 group vs. HER2 score 1+ group,  $P = 0.339$ ; HER2 score 0 group vs. HER2 score  $\geq 2+$  group,  $P = 0.004$ ; HER2 score 1 group vs. HER2 score  $\geq 2+$  group,  $P = 0.034$ .

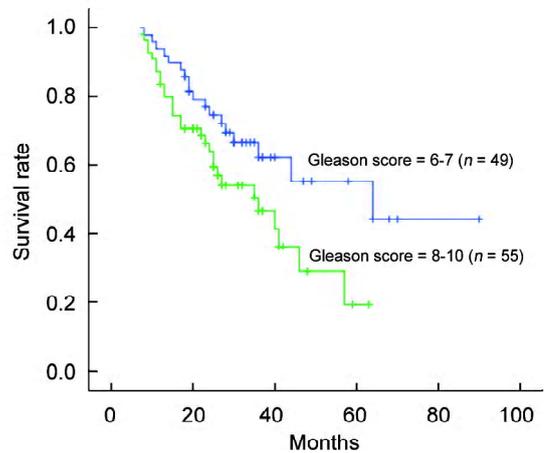


Figure 4. Prostate cancer specific survival curve for Gleason scores of 6–7 and 8–10 groups. Gleason score = 6–7 group vs. Gleason score = 8–10 group,  $P = 0.032$ .

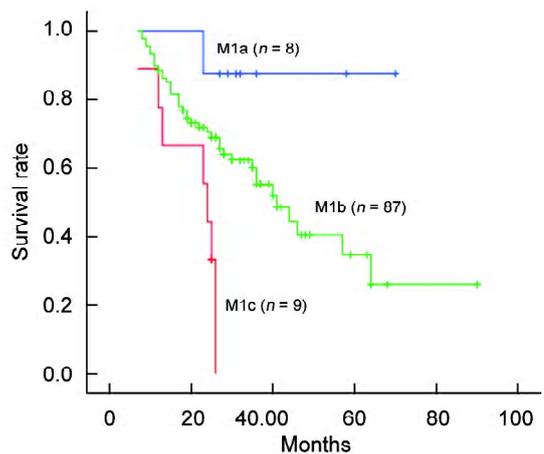


Figure 5. Prostate cancer specific survival curve for substage of M1a, M1b and M1c groups (M1a, non-regional lymph node(s) metastasis; M1b, bone(s) metastasis; M1c, other site(s) metastasis). M1a group vs. M1b group,  $P = 0.076$ ; M1a group vs. M1c group,  $P = 0.005$ ; M1b group vs. M1c group,  $P = 0.007$ .

However, for the majority of patients, the median duration of response is only 18–24 months [11]. Once hormone refractory disease is documented, treatment options are limited and the prognosis is extremely poor [11]. The mechanisms responsible for the development of androgen independence are not yet completely understood [4]. Recently, several experimental studies have proposed that androgen receptor (AR) gene mutation and amplification and alterations in growth factor activated

Table 3. Cox proportional hazards regression univariate and multivariate analysis of risk factors predicting prostate cancer specific survival. M1a, non-regional lymph node(s) metastasis; M1b, bone(s) metastasis; M1c, other site(s) metastasis.

Factor	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% confidence interval)	P-value	Hazard ratio (95 % confidence interval)	P-value
Age range	0.643 (0.362–1.143)	0.132	0.858 (0.462–1.593)	0.628
< 70 years				
≥ 70 years				
Gleason score	1.899 (1.041–3.464)	0.037	1.293 (0.664–2.518)	0.450
6–7				
8–10				
Serum PSA level (ng/mL)	1.646 (0.889–3.046)	0.113	1.340 (0.741–2.515)	0.363
< 100				
≥ 100				
Substage of metastatic PCa	3.218 (1.589–6.518)	0.001	2.746 (1.287–5.861)	0.009
M1a				
M1b				
M1c				
HER2 staining score	1.628 (1.093–2.588)	0.018	1.592 (1.025–2.474)	0.039
0				
1+				
≥ 2+				

pathways might play important roles in the progression to androgen independent and hormonal refractory prostate cancer [4,12]. The results of our previous studies indicate that there is a functional crosstalk between growth factors and growth factor receptors of the EGF receptor family and AR signaling pathways [12]. Dual blockade of AR function with the anti-androgen hydroxyflutamide and EGF receptor superfamily mediated signal transduction with the anti-EGF receptor monoclonal antibody C225, and the anti-HER2 receptor monoclonal antibody trastuzumab (Herceptin) significantly enhanced growth inhibition of the MDA PCa 2a cells [12]. Craft *et al.* [6] also found HER2 to activate the AR pathway in the absence of ligand and to synergize with low levels of androgen to increase AR signaling in a prostate cancer cell model. Collectively, these results strongly supported a role for HER2 expression in the development and progression of prostate cancer. Therefore, we designed the current study using only Chinese metastatic prostate cancer patients to determine the usefulness of HER2 status as a marker for predicting the patient outcome after MAB therapy.

The methods currently available for analyzing HER2

status in formalin-fixed paraffin embedded tissues include protein overexpression analysis by IHC and gene amplification analysis by FISH. HER2 protein overexpression detected by IHC is observed in ~30% of invasive breast carcinomas, which correlates with the HER2 gene amplification detected by FISH [5]. In prostate cancer, the situation is more complicated. Some studies have found that the HER2 gene amplification status detected by FISH correlates with HER2 protein expression detected by IHC [13]. However, more and more recently published studies have found no HER2 gene amplification in any stage of prostate cancer, even in those with HER2 protein overexpression [7, 10, 14]. One study using the Quantum Appligene probe technique confirms that none of 117 prostate cancer patients had true HER2 gene amplification [15]. Therefore, in the present study we used the economical IHC method to analyze the HER2 protein expression in each case and FISH was used for further investigation in those with HER2 IHC staining scores of 2+ or 3+.

In our patients, we found a correlation between the HER2 IHC staining intensity and the tumor Gleason score, which is consistent with previous studies [8, 16–18].

More importantly, our study suggests that in Chinese metastatic prostate cancer patients HER2 protein expression of the primary lesion at diagnosis influences the patient outcome. Although only 9.6% (10/104) patients in this study had an HER2 IHC score  $\geq 2+$ , their chance of dying from prostate cancer was significantly higher than those with HER2 scores of 0 or 1+. The only published report that evaluates the prognostic value of HER2 protein expression in metastatic prostate cancer patients comes from Japan [18]. In that study, the authors consider the HER2 score  $\geq 1+$  as protein overexpression and they find that the 3-year period causes specific survival rate in HER2 score  $\geq 1+$  group to be significantly lower than that in the HER2 score 0 group [18]. From our perspective, an HER2 IHC score 1+ should not be considered as protein overexpression. First, HER2 is a kind of transmembrane tyrosine kinase receptor that normally regulates cell growth, survival, adhesion, migration, differentiation and other cellular responses, and is expressed at low levels in normal epithelial cells, including prostate epithelial cells [7]. Second, in breast cancer patients, the HER2 IHC score 1+ is considered negative for HER2 protein overexpression [5, 17]. Third, in our Chinese metastatic prostate cancer patients, there was no difference in the cancer specific survival rate between the HER2 score 0 group and the HER2 score 1+ group (Figure 3). Moreover, multivariate Cox proportional hazards regression analysis showed that HER2 protein expression intensity was an independent predictor of prostate cancer-related death in this study (Table 3). However, our data suggest that the Gleason score is not an independent prognostic factor in our Chinese metastatic prostate cancer patients. Previous studies have also found that the Gleason score of the primary tumor has limited prognostic value in metastatic prostate cancer patients [19]. Therefore, to evaluate the HER2 protein expression is of particular importance and we recommend that HER2 IHC scores  $\geq 2+$  should be defined as HER2 protein overexpression in prostate cancer and used as an indicator for poor prognosis in patients with metastatic lesions.

In the present study, 12 patients underwent TURP to relieve urinary obstruction at least 3 months after initiation of MAB therapy. The HER2 protein expression intensity increased in 33.3% (4/12) of TURP specimens. Although statistical analysis did not show a significant increase in the HER2 protein expression intensity in our patients after a period of MAB therapy (Table 2), this phenomenon suggested that the expression of HER2 pro-

tein might increase within some individual patients after a period of hormonal therapy. Because these data came from the limited 12 matched pairs, the results should be considered as preliminary and we need to increase patient numbers to draw definite conclusions in the future. Previously, some researchers have also found that the expression of HER2 is higher in prostate cancers from patients who are treated with MAB before undergoing surgery compared with untreated patients, whereas the highest level of expression is found in patients who have developed androgen independent prostate cancer [14, 16]. A recently published experiment study by Berger *et al.* [20] provided an explanation for this phenomenon. They found that the induction and activation of HER2 in prostate cancer cells occurs in an androgen-depleted environment or as a result of AR inactivation, promoting survival and subsequent proliferation of ablation-resistant prostate cancer cells [20]. Although androgen ablation promotes G<sub>1</sub> phase arrest and/or apoptosis of many androgen dependent prostate cancers, a subset of cells survives and activates and overexpresses HER2 following androgen ablation [20]. Therefore, our study and others provide the biochemical rationale to target HER2 in hormone refractory prostate cancer. Trastuzumab (Herceptin, Genentech), a humanized monoclonal antibody that binds to the extracellular juxtamembrane domain of HER2 and inhibits the proliferation and survival of HER2-dependent tumors, is approved by the Food and Drug Administration for patients with invasive breast cancers that overexpress HER2 [5]. Clinical trails studying the efficacy of trastuzumab in patients with metastatic hormone refractory prostate cancer have been attempted [21]. One study using trastuzumab as a single agent in treating hormone refractory prostate cancer has shown relatively low efficacy [21]. Nevertheless, given the small number of patients in that study, further investigation is warranted.

We did not find HER2 gene amplification in any of the 14 specimens with HER2 IHC score  $\geq 2+$ . The only limitation of the present study was that we did not investigate the HER2/neu gene amplification status by FISH in HER2 IHC scores 0 and 1+ specimens for economic reasons. Theoretically, all these patients may obtain negative results. Currently, in prostate cancer, whether gene amplification of the HER2 locus plays a significant role in HER2 protein overexpression is uncertain. Some recently published studies also suggest that no HER2 gene amplification is found at any stage of prostate cancer [7,

10, 14]. Therefore, in prostate cancer, HER2 protein expression might be regulated mainly at the transcription and post-translation level, and HER2 protein expression analysis by IHC is more sensitive and more important than gene amplification analysis by FISH for evaluation of HER2 status in these patients.

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