

## RESEARCH ARTICLE

# Possible Role of HER-2 in the Progression of Prostate Cancer from Primary Tumor to Androgen Independence

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### Abstract

**Background:** The expression of HER-2 in prostate cancer has been linked to disease progression. We analysed the presence of HER-2 expression in primary tumors in men undergoing radical prostatectomy, its association with clinical and pathological findings, and its expression in secondary circulating prostate cells (CPCs) during follow up, as well as links with biochemical failure and the effects of androgen blockade. **Materials and Methods:** Consecutive men undergoing radical prostatectomy for histologically confirmed prostate cancer were analyzed. HER-2 expression in the primary tumor was assessed using the HercepTest®, CPCs were identified from blood samples using standard immunocytochemistry with anti-PSA and positive samples with the HercepTest® to determine HER-2 expression. The influence of HER-2 expression on the frequency of biochemical failure and effects of androgen blockade was determined. **Results:** 144 men with a mean age of 64.8±10.3 years participated, with a median follow up of 8.2 years. HER-2 was expressed in 20.8% of primary tumors; it was associated with vascular infiltration and older age, but not with other clinical pathological findings. Some 40.3% of men had secondary CPCs detected, of which 38% expressed HER-2. Men CPC (+) had a higher frequency of biochemical failure, but there was no difference in HER-2 expression of CPCs with the frequency of biochemical failure. After androgen blockade, men with HER-2 (+) positive secondary CPCs had a higher frequency of disease progression to castrate resistant disease. **Conclusions:** HER-2 plays a dual role in the progression of prostate cancer; firstly it may increase the potential of tumor cells to disseminate from the primary tumor via the blood by increasing vascular infiltration. In the presence of androgens, there is no survival advantage of expressing HER-2, but once biochemical failure has occurred and androgen blockade started, HER-2 positive cells are resistant to treatment, survive and grow leading to castration resistant disease.

**Keywords:** Prostate cancer - HER-2 - circulating prostate cells - biochemical failure - castrate resistant

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### Introduction

There is evidence linking the expression of HER-2 in prostate cancer to disease progression and the development of androgen independent disease. HER-2 is a member of the ErbB family of receptor tyrosine kinases and plays a crucial role in growth, differentiation, and motility of normal and cancer cells. HER-2 has been proposed as a survival factor for prostate cells in the absence of androgens, possibly by activating the androgen receptor (Craft et al., 1999; Signoretti et al., 2000; Osman et al., 2001). In hormone-naïve patients, whether in patients undergoing observation, or post-treatment with or without biochemical failure, the expression of HER-2 is infrequent both in the original tumor, circulating prostate cells (CPCs) and micro metastasis (Osman et al., 1999; Murray et al., 2011), whereas patients treated with androgen blockade have significantly increased levels of HER-2 expression

in both the original tumor, CPCs and micro metastasis (Osman et al., 1999; Murray et al., 2011). There is a suggestion that HER-2 may have a role in prostate cancer development, however its expression is variable, ranging between 0 to 100% depending on the HER-2 assay used (Ratan et al., 2003) and gene amplification of the HER-2 gene ranges from 0 to 53% (Liu et al., 2001; Montironi et al., 2006). Despite this discrepancy, there is still a consistent association between HER-2 over expression and a higher risk tumor recurrence and death in men with prostate cancer according to a meta-analysis (Neto et al., 2010). Secondary CPCs are associated with a seven fold increase of biochemical failure after radical prostatectomy and CPC detection using standard immunocytochemistry is able to identify a high risk group for biochemical failure before there is a rise in the serum PSA (Murray et al., 2013).

We present a prospective study of the detection of the

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expression of HER-2 in the primary tumor, the expression of HER-2 in secondary circulating prostate cells (CPCs) after radical prostatectomy, its association with the clinical pathological findings and its influence on the occurrence of biochemical failure and the effect of androgen blockade.

## Materials and Methods

### Patient selection

From January 2002 to December 2012 patients were recruited to the study and follow up continued until December 2014. All men treated by radical prostatectomy at the author's institution were invited to participate. Clinical-pathological findings were recorded from the surgical piece; Gleason score; total serum PSA pre-surgery, the presence or absence of lymphatic, vascular and perineural infiltration; capsular infiltration, presence or absence of positive margins.

a) HER-2 expression in the primary tumor: sections of the surgical piece with prostate cancer were selected for the determination of HER-2 expression. This was evaluated using the FDA approved immunocytochemical kit HercepTest (DAKO, USA). HER-2-positive patients were defined according to the criteria of Osman et al. (2001) as 2+ and 3+ staining in more than 10% of prostate cancer cells.

b) detection of secondary CPCs and HER-2 expression: blood samples from consecutive prostate cancer patients were prospectively collected for the purpose of detecting CPCs and evaluating whether these cells were correlated with clinical outcomes. Samples were taken from men at least three months after surgery and considered to be without evidence of disease, defined as being bone scan negative and a serum PSA <0.20ng/ml. Blood sampling was repeated at four monthly intervals for total serum PSA and secondary CPCs until biochemical failure, defined as a total serum >0.2ng/ml or until the end of the study period. If biochemical failure occurred patients were treated initially with flutamide, 250mg po eight hourly and then with triptorelina 11mg im three monthly. In these patients blood samples were taken four monthly for total serum PSA and CPC determination.

c) Identification of secondary CPCs: Slides were processed within 1 hour of fixation and incubated with anti-PSA clone 28A4 (Novocastra Laboratory, UK) in a concentration of 2.5 µg/ml for 1 hour at room temperature and identified using a detection system based on alkaline phosphatase-antialkaline phosphatase (LSAB2 DAKO, USA) with new-fuschin as the chromogen. To permit the rapid identification of positive cells there was no counter staining with Mayer's hematoxilin. Levisamol (DAKO, USA) was used as an inhibitor of endogenous alkaline phosphatase. Positive and negative controls were processed in the same way. Definition of secondary CPCs using the criteria of ISHAGE was used to identify immunostained cells (Borgen et al., 1999) (Figure 1, 2). A sample was classified as CPC positive if one PSA positive cell was detected.

HER-2 immunostaining; Samples positive for CPCs underwent a second process using HercepTest®, HER-2 staining was classified as negative for 0 and 1+ staining

and positive for 2+ and 3+ staining<sup>2</sup> (Figure 3). A sample was classified as HER-2 positive if there was one PSA positive cell staining HER-2 2+ or 3+.

Samples were analyzed at low power and photographed at a magnification of 400X with a Samsung Digimax D73 digital camera and processed using Digimax for Windows. The immunocytochemical processing and evaluation was carried out by a single person blinded to the clinical details.

Planned analysis of the results:

Expression of HER-2 in the primary tumor was determined, a positive test was >10% of prostate cancer cells expressing HER-2 with a score of +2 or +3; a second group, classified as negative with 1-9% of cancer cells expressing HER-2 with a score of +2 or +3 and a third group classified as negative with no cell positive for HER-2 expression. Clinico-pathological findings were compared with the presence or absence of secondary CPCs, and the expression of HER-2 on secondary CPCs. These three groups, CPC (+) HER-2 (+); CPC (+) HER-2 (-) and CPC (-) were followed up, and the number of men progressing to biochemical failure determined. After androgen blockade; these men continued to be followed up until the serum PSA started to rise again, implying resistance to androgen blockade.

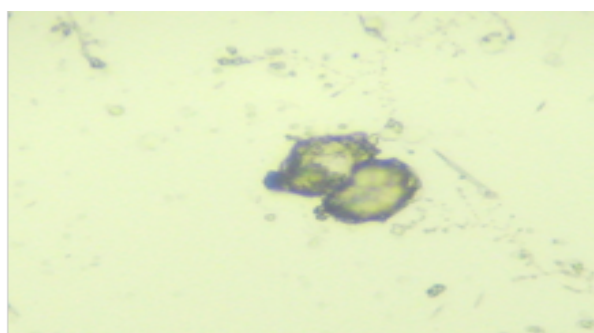


Figure 1. Leukocytes PSA (-)

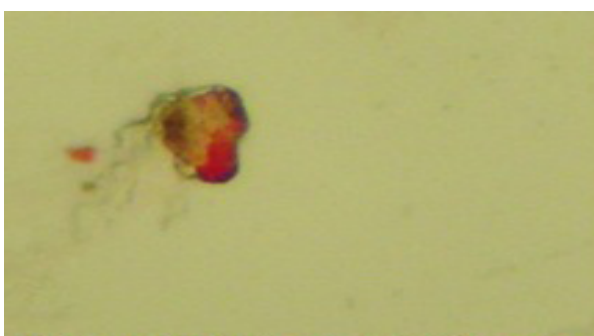


Figure 2. CPC PSA (+) Red



Figure 3. CPC PSA (+) HER-2 (+) (Black)

Statistical Analysis of the results: Descriptive statistics were used to compare demographic and disease characteristics of patients with and without biochemical failure. Univariate comparisons were tested using Chi squared and Kaplan Meier methods were used to compare the unadjusted free from biochemical failure of patients with and without CPCs detected. Age, pathological stage (organ confined, non organ confined), pathological grade, margin status (positive, negative), capsule compromise (positive, negative), peri-neural, vascular and lymphatic infiltration (positive, negative) were compared with the presence/ absence of CPCs and the expression of HER-2 and with and without biochemical failure. Men undergoing androgen blockade were analyzed using Chi-squared or Fisher exact test for disease progression.

## Results

144 men with a mean age of  $64.8 \pm 10.3$  years and a median serum total PSA of 6.32ng/ml (inter-quartile range 4.73-8.10ng/ml) at the time of radical prostatectomy participated in the study. Median follow up was 8.2 years (IQR 6.2-10.3) 30 (20.8%) prostate cancers were positive for HER-2 expression, of the 114 cancers classified as HER-2 negative; 76 (67%) had between 1-9% of cells expressing HER-2 and 38 (33%) not expressing HER-2. There was no association of HER-2 expression with Gleason score, pathological stage or total serum PSA. However, men with tumors expressing HER-2 were significantly older than men with HER-2 negative tumors or those classified as negative (Table 1).

a) CPC detection and HER-2 expression: 58 (40.3%) of men had secondary CPCs detected, of which 22/58 (38%) expressed HER-2. Of the patients with HER-2 (+) CPCs, 19 were from HER-2 (+) primary tumors, 3 from HER-2 negative (but with 1-9% HER-2 expression) primary tumors and none from HER-2 negative (0% HER-2 expression) primary tumors (see Table 2).

Men with HER-2 (+) primary tumors had a higher frequency of CPCs detected, 22/30 (73%) versus 36/114 (32%) of men with HER-2 (-) ( $p=0.0001$  Chi squared, Odds Ratio 5.95 (95% CI 2.42-14.66). Not all men with HER-2 (+) tumors had HER-2 (+) secondary CPCs, similarly men with primary tumors classified as HER-2 (-) (1-9% expression HER-2) had HER-2 (+) secondary CPCs.

Men with HER-2 (+) CPCs were significantly older than men with HER-2 (-) CPCs,  $74.1 \pm 7.8$  years versus  $69.5 \pm 7.9$  years ( $p=0.034$ ) respectively, but median PSA levels were not different 6.11ng/ml (IQR 5.09-14.63) versus 6.14ng/ml (IQR 5.05-8.11) ( $p=0.054$ ). With respect to clinical-pathological characteristics of the surgical specimen, only perivascular invasion was significantly higher in men with HER-2 (+) CPCs ( $p=0.024$ ) (Table 3).

c) Biochemical Failure: Significantly more men 31/58 (53%) with secondary CPC (+) suffered biochemical failure in comparison with 8/86 (9%) of men CPC (-) ( $p<0.001$ ). However, there was no significant difference between the frequency of biochemical failure between men secondary CPC (+) HER-2 (+) and men secondary CPC (+) HER-2 (-); 13/22 (59%) versus 18/36 (50%)

( $p=0.50$ ) respectively. A Kaplan Meier curve also failed to show a significant difference in biochemical failure free survival between CPC (+) HER-2 (+) and CPC (+) HER-2 (-) men (Figure 4) as determined by log-rank analysis.

**Table 1. Clinico-Pathological Features and Primary Tumor HER-2 Expression**

	HER-2 (+) ( $\geq 10\%$ cells +)	HER-2 (-) (1-9% cells +)	HER-2 (-) (0% cells +)	
N° Patients	30	76	38	
Age $\pm$ SD	73.9 $\pm$ 7.8*	69.4 $\pm$ 8.6**	61.6 $\pm$ 5.9**	* $p<0.01$ ** $p<0.01$
PSA $\pm$ IQR	6.11 (4.76-12.62)	6.14 (5.01-8.61)	5.93 (4.15-9.34)	NS
Gleason $\leq 6$	18	50	25	NS
$\geq 7$	12	26	13	
pT2	22	53	30	NS
pT3	8	23	8	

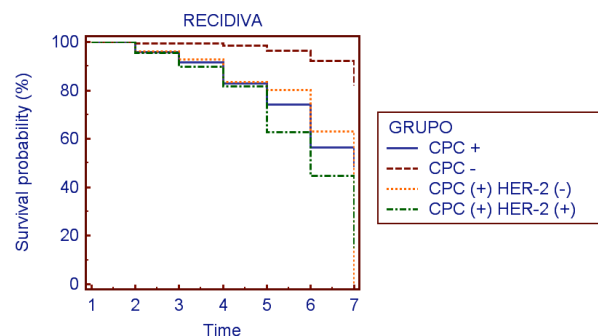
**Table 2. HER-2 Expression in Secondary CPCs according to HER-2 Expression in the Primary Tumor**

	HER-2(+) ( $\geq 10\%$ cells+)	HER-2(-) (1-9% cells+)	HER-2 (-) (0% cells+)	
N° patients	30	76	38	144
CPC (+) HER-2 (+)	19	3	0	22
CPC (+) HER-2 (-)	3	21	12	36
CPC (-)	8	52	26	86

**Table 3. Association of CPC HER-2 Expression and Clinical Pathological Findings in the Surgical Specimen**

	CPC HER-2 (+)	CPC HER-2 (-)	p value
margin (+)	11	13	
margin (-)	11	23	$p=0.30$
ECE (+)	17	26	
ECE (-)	7	10	$p=0.57$
PN (+)	15	23	
PN (-)	7	13	$p=0.74$
PV (+)	14	12	
PV (-)	8	24	$p=0.024$
PL (+)	8	12	
PL (-)	14	24	$p=0.81$
Gleason $\leq 6$	13	24	
Gleason $\geq 7$	9	12	$p=0.56$

ECE= extra-capsular extension; PN= perineural infiltration; PV= perivascular infiltration; PL=perilymphatic infiltration]



**Figure 4. Kaplan-Meier Curves for CPC Positive and Negative Men with Biochemical Failure Free Survival.** Log Rank: CPC (+) versus CPC (-)  $p<0.0001$ ; CPC (+) HER-2 (-) versus CPC (+) HER-2 (-)  $p=0.47$

d) Effect of androgen blockade: All 39 men with biochemical failure underwent androgen blockade, with flutamide 250mg 8 hourly followed by depot injections of triptorelina 11.5mg im. All men had a decrease in serum total PSA within 5 months of treatment independent of CPC status and HER-2 expression. With a median follow-up of 3 years (IQR 2-4), 9/13 (69%) of men CPC (+) HER-2 (+); 3/18 (17%) of men CPC (+) HER-2 (-) and 1/8 (13%) of men CPC (-) became castrate resistant during follow up, as shown by increasing serum PSA levels. Significantly more men CPC (+) HER-2 (+) became castrate resistant compared with CPC (+) HER-2 (-) men ( $p=0.008$  relative risk 5.54 (95% CI 0.86-35.9) and compared with men CPC (-) ( $p=0.008$  relative risk 4.15 (95% CI 1.39-12.4). There was no significant difference between men CPC (+) HER-2 (-) and CPC (-) men.

## Discussion

HER-2 is a 185-kDa Transmembrane tyrosine kinase receptor and belongs to the epidermal growth factor family (Wen et al., 2000). HER-2 is located on chromosome 17q21 and HER-2 signaling promotes cells proliferation through the RAS-MAPK pathway and inhibits cell death through the phosphatidylinositol 3'-kinase-AKT pathway (Hudis, 2007). Pre-clinical studies suggest that HER-2 expression plays a role in prostate cancer progression. Using a prostate cancer xenograft model it has been shown that androgen independent cancer cells have a higher expression of HER-2 than androgen dependent cancer cells, and that forced over-expression of HER-2 convert's androgen dependent cells into androgen independent cells (Craft et al., 1999). In the clinical, HER-2 has been proposed as a survival factor for prostate cells in the absence of androgens, possibly by activating the androgen receptor (Craft et al., 1999; Signoretti et al., 2000; Osman et al., 2001). The proposed mechanism for the role of HER-2 in hormone escape is that it activates androgen receptor phosphorylation (via the MAPK or AKT pathways) which in turn maintains the androgen receptor integrity and thus its function in the absence of testosterone (Yeh et al., 1999; Wen et al., 2000). In prostate cell models it has been reported that, in androgen independent cell lines, HER-2 expression and AKT activation are increased, and the use of the anti-HER-2 drug trastuzumab can reverse this (Guyader et al., 2012). The use of androgen blockade has been shown to increase HER-2 expression and suggested that this therapy eliminates HER-2 negative cells; thus decreasing the serum PSA in men with biochemical failure (Guyader et al., 2012).

The expression of HER-2 in the primary tumor was positive ( $\geq 10\%$  expressing HER-2) in 20.8% of cases, which is similar to that reported by other authors (Zahir et al., 2014). There was no association with the Gleason score or pathological stage, which is similar to some reports (Lara et al., 2002; Zahir et al., 2014). However other studies have reported an association with the Gleason score, HER-2 expression being higher in patients with a Gleason score  $>7$  (Neto et al., 2010).

In patients who had secondary CPCs expressing HER-2 there was an increased risk of vascular infiltration

in the original tumor, as compared with men with CPCs HER-2 negative. Patients with HER-2 positive CPCs were older at the time of diagnosis. There were no differences between men with HER-2 positive and negative CPCs with regards to the other clinical parameters. This suggests that HER-2 (+) cancer cells have an increased ability to disseminate to the vascular structures and then into the circulation where they can be detected as CPCs. In breast cancer it has been reported that HER-2 expression in bone marrow micro-metastasis is higher than the original tumor and is associated with early dissemination and metastasis (Fehm et al., 2010). In patients prior to radical cystectomy for urothelial carcinoma of the bladder, HER-2 expression in circulating tumor cells was higher than in the tumor original (Rink et al., 2012). Together these findings would seem to suggest that HER-2 positive cells in the original tumor have a higher capacity of vascular dissemination than HER-2 negative cells. There were more patients with HER-2 (+) CPCs than patients with a primary tumor classified as HER-2 (-). This can be explained by the fact there are a group of men with primary tumors classified as HER-2 (-) but with cancer cells HER-2 (+) present in the tumor, with the same potential for vascular dissemination as men classified as having a primary tumor HER-2 (+). Thus in terms of vascular dissemination a cutoff value of  $>10\%$  cells expressing HER-2 in the primary tumor to define positivity may be misleading. That men 9% of men with a primary tumor HER-2 (+) were CPC negative suggests that other factors are involved in vascular dissemination.

Dissemination from the primary tumor is an essential process for the development of metastasis but is not by itself sufficient to complete the metastatic pathway (Weiss, 1990). However the presence of CPCs after radical prostatectomy implies the presence of distant micrometastasis or remnant local disease. There was no difference in the frequency of biochemical failure between men with HER-2 (+) and HER-2 (-) CPCs, however with the implementation of androgen blockade, men with HER-2 (+) CPCs had a significantly higher rate of developing castrate resistant prostate cancer. This suggests, that serum PSA decreases with androgen blockade as a result of the death of HER-2 (-) cells, the HER-2 (+) are resistant to androgen blockade, survive and proliferate which is reflected in the later increase in serum PSA. This process has previously been reported in bone marrow micrometastasis (4) and could explain why the expression of HER-2 has an adverse effect on prognosis and recurrence after endocrine treatment (Shariat et al., 2007; Siampanopoulou et al., 2013)

In conclusion, The results of this study suggest that HER-2 plays a dual role in the progression of prostate cancer; firstly increases the potential of tumor cells to disseminate from the primary tumor via the blood by increasing vascular infiltration. Thus these tumor cells are found distant from the operation site of radical prostatectomy and escape primary treatment. In the presence of androgens, there is no survival advantage of expressing HER-2, the risks of biochemical failure being the same as those patients with HER-2 negative CPCs. However, once biochemical failure has occurred

and androgen blockade started, HER-2 positive cells are resistant to treatment, survive and grow leading to castrate resistant disease.

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