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*Original Article*

# Carbonic Anhydrase IX Expression and Outcome after Radiotherapy for Muscle-invasive Bladder Cancer

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**ABSTRACT:**
***Aims:*** **Carbonic anhydrase IX (CA IX) expression has been described as an endogenous marker of hypoxia in solid neoplasms. Furthermore, CA IX expression has been associated with an aggressive phenotype and resistance to radiotherapy. We assessed the prognostic significance of CA IX expression in patients with muscle-invasive bladder cancer treated with radiotherapy.**
***Materials and methods:*** **A standard immunohistochemistry technique was used to show CA IX expression in 110 muscle-invasive bladder tumours treated with radiotherapy. Clinicopathological data were obtained from medical case notes.**
***Results:*** **CA IX immunostaining was detected in 89 (~81%) patients. Staining was predominantly membranous, with areas of concurrent cytoplasmic and nuclear staining and was abundant in luminal and perinecrotic areas. No significant correlation was shown between the overall CA IX status and the initial response to radiotherapy, 5-year bladder cancer-specific survival or the time to local recurrence.**
***Conclusions:*** **The distribution of CA IX expression in paraffin-embedded tissue sections seen in this series is consistent with previous studies in bladder cancer, but does not provide significant prognostic information with respect to the response to radiotherapy at 3 months and disease-specific survival after radical radiotherapy. Sherwood, B. T. *et al*. (2007). *Clinical Oncology* 19, 777–783**



**Key words:** Bladder cancer, carbonic anhydrase IX, hypoxia, radiotherapy

## Introduction

About 30% of bladder tumours are muscle invasive at presentation and are therefore associated with a significant risk of metastasis and a poor prognosis. Radical radiotherapy is the cornerstone of treatment regimens aimed at bladder preservation. However, a complete local response is seen in only 50% of cases. Predictive information regarding the probable response of a bladder tumour to radiotherapy would be of enormous benefit in enhancing patient selection for bladder preservation.

An association between tumour hypoxia and resistance to treatment with ionising radiation has long been recognised. In other solid tumours, polarographic needle measurements of hypoxia correlate with increased metastatic potential, resistance to radiotherapy and an adverse prognosis [1–3]. However, bladder carcinomas are not readily accessible to microelectrodes and alternative strategies aimed at hypoxia measurement need to be assessed.

The transcriptional complex hypoxia-inducible factor-1 (HIF-1) is recognised as a key mediator of gene expression in hypoxic tumours. Hypoxic induction of the carbonic anhydrase genes CA9, CA12 and corresponding proteins (CA IX, CA XII), has been shown to be HIF-1 dependent [4]. In tumour cells, these enzymes are pivotal in maintaining the intracellular pH at physiological levels. The overall effect of CA activity is the relative acidification of the extracellular space. This has important ramifications in promoting further tumour growth and invasion. CA IX expression has been reported as an endogenous surrogate marker of hypoxia in solid neoplasms. In cervical carcinoma, for example, CA IX expression correlates well with polarographic measurements of tumour oxygen tension [5]. More recently, CA IX expression has been associated with poor prognosis in non-small cell lung cancer [6] and has been associated with a poor response to chemoradiotherapy in head and neck cancer [7].

In bladder cancer, CA IX immunostaining is attractive as a marker of hypoxia, as it is non-invasive and does not require systemic administration, compared with polarographic needle measurements and pimonidazole, respec-

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tively. Significant correlations have been observed between CA IX expression and pimonidazole levels in bladder cancer [4]. However, there is no clear consensus as to the prognostic value of CA IX immunostaining in this disease. Turner *et al*. [8] studied the distribution of vascular endothelial growth factor mRNA (by in situ hybridisation) and CA IX expression in 22 bladder cancers of varied pathological stages. Co-localisation of vascular endothelial growth factor and CA IX expression was observed, with both being expressed predominantly on luminal surfaces and in perinecrotic areas. The expression of both factors was greater in superficial disease compared with invasive disease. The investigators went further, to study the relationship between the expression of CA IX and clinical outcome in 49 patients with superficial bladder cancer. CA IX expression was not predictive of clinical outcome. Hoskin *et al*. [9] investigated GLUT1 and CA IX as endogenous markers of hypoxia and their relationship to outcome in a retrospective series of 64 bladder cancer patients treated with radical radiotherapy with carbogen and nicotinamide (ARCON). GLUT1 and CA IX staining were found to be independent predictors for overall and disease-specific survival, but not for local control or metastasis-free survival. A prospective study was also reported in which pimonidazole, GLUT1 and CA IX staining was compared in 21 patients with bladder cancer. A good correlation was reported between CA IX/GLUT1 expression and pimonidazole staining. More recently, CA IX expression has been studied in 57 patients with superficial or invasive disease [10]. Again, significantly more superficial bladder cancers expressed CA IX strongly. However, no significant association between CA IX staining and survival was established in either superficial or invasive disease.

In the present study, we evaluated CA IX expression in invasive bladder cancer using standard immunohistochemistry. We determined the prognostic significance of tumour CA IX expression in patients treated with radiotherapy; the primary end points being the initial response to radiotherapy and survival (bladder cancer specific) and a secondary end point being local recurrence.

## Materials and Methods

### *Study Population*

Ethical approval was obtained for the study of archival paraffin-embedded tissue sections from 110 patients with pathological stage T2—T3 bladder cancer. The same study population was used in a recent immunohistochemical study to show that epidermal growth factor receptor status predicts local response to radical radiotherapy in muscle-invasive bladder cancer [11]. Therefore, we are confident that the size of the study population of the present study has the power to detect any differences, to the extent of the previous study, should they exist.

All patients were treated with 6 or 8 Mv X-rays between 1992 and 1997. The most commonly used regimen (77 patients) was 60 Gy in 30 fractions given over 42 days. Twenty-five patients were treated with 50—55 Gy in 20 fractions. Others received varying doses between 45 and 64 Gy in 20—32 fractions. Treatment was given to the bladder only, with a 1—1.5 cm margin. Most patients were planned using a cystogram and cystoscopic findings were taken into account in deciding the treatment volume. In the last 2 years of the study, patients were planned on computed tomographic images. Of the specimens, 91 (82.7%) were from men and 19 (17.3%) were from women. Staging was based on biopsy reports from the initial transurethral resection of the bladder tumour (TURBT). The clinicopathological data are summarised in Table 1. Hospital notes were reviewed to determine the following clinical outcomes; the initial response to radiotherapy, local and distant tumour recurrence rates and survival. The initial response to

**Table 1 — Summary of clinicopathological data**

| Characteristic | *n* | % |
|---|---|---|
| No. of patients | 110 | 100 |
| Age at diagnosis (years) | | |
| 50—59 | 8 | 7.3 |
| 60—69 | 25 | 22.7 |
| 70—79 | 53 | 48.2 |
| 80—89 | 24 | 21.8 |
| Gender | | |
| Male | 91 | 82.7 |
| Female | 19 | 17.3 |
| Histological type | | |
| Transitional cell carcinoma (TCC) | 106 | 96.4 |
| Squamous cell carcinoma | 4 | 3.6 |
| Tumour grade | | |
| 2 | 16 | 14.5 |
| 3 | 93 | 84.5 |
| Unknown | 1 | 0.9 |
| Tumour stage (clinical) | | |
| T1—2 | 5 | 4.5 |
| T2—3 | 48 | 44.7 |
| T3—4 | 34 | 30.9 |
| T4 | 7 | 6.4 |
| Not recorded | 16 | 14.5 |
| Pre-treatment ureteric obstruction | | |
| Yes | 64 | 58.2 |
| No | 27 | 24.5 |
| Not assessed | 19 | 17.3 |
| Previous non-muscle-invasive tumour | | |
| Yes | 29 | 26.4 |
| No | 81 | 73.6 |
| Radiation therapy | | |
| Mean total dose (range, standard deviation) | 57.9 (49—64 ± 4.3 Gy) | |
| Modal fraction size (range, standard deviation) | 2 (2.0—2.5 ± 0.2 Gy) | |
| Modal fraction number (range, standard deviation) | 30 (20—32 ± 3.5) | |
| Mean duration of treatment (range, standard deviation) | 45.1 (27—87 ± 9 days) | |