Pergamon

*European Journal of Cancer* Vol. 31A, Nos 13/14, pp. 2243–2247, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0959-8049/95 $9.50+0.00

0959-8049(95)00443-2

## Original Paper

# p53 Overexpression as a Prognostic Factor for Advanced Stage Bladder Cancer

M.A. Kuczyk,[1] C. Bokemeyer,[3] J. Serth,[1] C. Hervatin,[1] M. Oelke,[1] K. Höfner[1], H.K. Tan[2] and U. Jonas[1]

Departments of [1]Urology, [2]Biostatistics and [3]Haematology/Oncology, Hannover Medical School, Hannover, Germany

**Overexpression of the *TP53* gene protein detected by immunohistochemistry appears to identify those patients with superficial bladder cancer at risk of the development of muscle invasive or metastatic disease. However, the role of p53 overexpression in patients with advanced or metastatic bladder cancer is not yet well established. In the present study, 44 specimens from 44 patients with advanced stage bladder tumours ($T_2$–$T_4$) undergoing radical cystectomy were investigated for different biological and clinical characteristics as possible prognostic factors: sex, age, depth of tumour infiltration, T-stage, histological grade, lymph node status, application of adjuvant systemic chemotherapy (MVAC), proliferative activity (staining for proliferating cell nuclear antigen (PCNA) by monoclonal antibody (PC10) as well as overexpression of the p53 oncoprotein (monoclonal antibody pAb 1801)). After a median follow-up of 22 months, 16 of the 23 patients (70%) with more than 40% of tumour cells stained positively for p53 (Group B) died from tumour progression compared with 7 of the 21 patients (33%) with less than 40% of tumour cells positive for p53. During univariate analysis, p53 overexpression ($P = 0.008$), staining for PCNA ($\geq$ 80% of cells positive) ($P = 0.01$) and tumour stage ($P = 0.01$) were significant prognostic factors for survival, among which p53 overexpression ($P = 0.023$) as well as T-stage ($P = 0.012$) remained independent significant predictors during multivariate analysis. Prospective studies are needed to confirm the independent prognostic potential of p53 overexpression in patients with advanced bladder cancer. The availability of more refined prognostic factors should assist decision making regarding the value of more aggressive treatment options, such as adjuvant or neoadjuvant chemotherapy, for prognostically defined subgroups of patients.**

**Key words: p53 tumour suppressor gene, muscle invasive bladder cancer, pathogenesis, prognostic factors, PCNA**

*Eur J Cancer*, Vol. 31A, Nos 13/14, pp. 2243–2247, 1995

## INTRODUCTION

TRANSITIONAL CELL carcinoma of the bladder is the fifth most common cancer in Western countries. With a continuously increasing incidence over the last few years, the actual annual incidence of bladder cancer has reached 16 to 20 per 100 000 population in the U.S.A. [1]. Approximately 80% of tumours are diagnosed as superficial cancers and only 10–20% are advanced stage disease or metastatic at primary diagnosis. Recurrences of superficial cancer will remain confined to the bladder wall in 70–80% of patients, but 20–30% of recurrent tumours will subsequently become muscle invasive and lead to metastatic disease [2, 3]. Approximately half the patients with muscle-invasive bladder cancer at initial diagnosis have already developed occult regional or distant metastases, with an extremely poor prognosis. For node-positive patients with locally advanced tumours, Skinner and associates [4] reported a 5-year survival rate of 36%, and Roehrborn and associates [5] reported a 3-year survival rate of between 18% ($N_2$) and 30% ($N_1$).

There is a considerable variance in survival times of patients with muscle invasive bladder tumours despite comparable tumour stages, grading and lymph node status, suggesting that biological different types of invasive bladder tumours with different clinical behaviour seem to exist, or that at least currently available prognostic factors are not sufficient to predict the aggressiveness of bladder tumours [1]. However, a prognostic factor which would allow the prediction of the clinical course of an individual patient with bladder cancer in addition to

Correspondence to M.A. Kuczyk.
Revised 5 May 1995; accepted 27 Jul. 1995.

established prognostic parameters, such as histopathological growth pattern (uni- or multifocal), tumour stage, grade and lymph node status, is still lacking.

For a variety of tumours, such as cancer of the colon, breast, lung and prostate [6–9], the detection of a mutational inactivation of the *TP53* tumour suppressor gene, resulting in an altered gene product, the p53 oncoprotein, has been identified as a prognostic factor for the clinical course of disease. Mutation of the *TP53* gene leads to a protein with altered configuration and a prolonged half life, and higher intracellular levels compared to the wild type protein, thereby allowing easy and reliable detection of this event by the use of immunohistochemical methods [10, 11].

In superficial bladder cancer ($T_a$,$T_1$), a strong corrrelation between overexpression of the p53 oncoprotein and the later development of muscle invasive disease with poor clinical outcome has been proposed [12–14]. However, the prognostic value of p53 gene protein overexpression for advanced stage bladder tumours is controversial [15–17].

The present study attempted to determine the prognostic value of p53 oncoprotein expression for patients with advanced stage bladder cancer. In 44 patients undergoing radical cystectomy for the treatment of muscle invasive bladder tumours, detection of the p53 protein was corrrelated with clinically important variables such as sex, age, tumour stage, grade, lymph node status, and proliferation rate determined by immunohistochemical staining for the "proliferating cell nuclear antigen" (PCNA), and finally the survival of the patients.

## PATIENTS AND METHODS

*Patients*

44 patients (14 females and 30 males) undergoing radical cystectomy for the treatment of muscle invasive bladder cancer were included. The median age of the patients was 68 years (range: 37–86 years). Following radical cystectomy, tumour specimens were classified as $T_2$ (8 patients), $T_3$ (22 patients) and $T_4$ tumours (14 patients) according to the TNM system. All tumours were histologically graded as $G_2$ or $G_3$. Patients underwent a bilateral lymph node dissection, followed by radical cystectomy and supravesical urinary diversion. In 8 patients with $T_4$ (5 patients: $N_1$; 3 patients: $N_2$) as well as in 5 patients with $T_3$ tumours ($N_1$: 3 patients, $N_2$: 2 patients) and in 3 patients with $T_2$ tumours ($N_1$), regional lymph node metastases were diagnosed during histopathological examination. In 2 patients, a single liver metastasis was surgically resected. All patients were considered disease free after radical tumour surgery. 10 patients (6 stage $T_4$ and 4 stage $T_3$) received 3–4 courses of adjuvant systemic chemotherapy (MVAC-regimen). Patients were followed by computed tomography (CT) scans of the abdomen and X-ray of the lungs, every 3 months during the first year after cystectomy and every 3–6 months thereafter. The median follow-up since radical cystectomy for all patients was 22 months (range: 1–57 months).

*Immunohistochemistry*

Formalin-fixed and paraffin-embedded tissue sections from the 44 bladder tumours were investigated for overexpression of the p53 oncoprotein by an immunohistochemical approach. p53 immunoreactivity was also studied in five biopsy specimens from normal bladder epithelium in non-tumour carrying patients.

Positive controls were represented by tumour specimens known to contain a mutational inactivation of the *TP53* tumour suppressor gene as detected by DNA-sequence analysis of the *TP53* gene. As an internal negative control for the staining procedure, each tumour in the study was incubated with non-immune mouse IgG instead of the primary antibody, followed by the identical procedure for the application of the secondary antibodies. Five biopsies from normal bladder epithelium and the normal mesenchymal cells within the tissue sections of the resected tumours served as biological negative controls. Following dewaxing, the tumour specimens were cut serially at 8 μm thickness and stained for p53 and PCNA by an identical immunohistochemical procedure, apart from the different primary antibodies.

*Immunohistochemistry for p53.* Slides of tumour tissue were first incubated with normal human serum at a dilution of 1:100 in Tris-buffered saline (TBS; 0.05 M, pH 7.6) to prevent non-specific binding of the first antibody. The specific primary antibody for the detection of p53 (pAB 1801/Dianova, D-2000 Hamburg, Germany) [18] was then added. The pAb 1801 mouse monoclonal antibody recognises a denaturation-resistant epitope in both mutant and wild-type p53 proteins and enables the detection of altered p53 proteins within the cell nucleus, due to their prolonged half-life caused by conformational changes as a result of the genetic mutation [11]. The pAb 1801 antibodies were applied at a dilution of 1:50 in TBS at room temperature for 1 h in a moist chamber.

After rinsing with TBS/0.1% TWEEN 20 (SERVA) for 10 min, the sections were incubated with a second monoclonal antibody of rabbit–anti-mouse specificity (Z 259, Dako, D-2000 Hamburg, Germany). This antibody was applied in a mixture of human serum and TBS (1:25) for 30 min diluted at 1:25. After a third rinsing with TWEEN 20/TBS, the APAAP (alkaline phosphatase–anti-alkaline phosphatase) complex (DAKO) was added in a dilution of 1:50 in TBS for 30 min. After a final rinsing with TWEEN 20/TBS, the red reaction product was obtained following the usual chemical reaction procedure. Finally, the slides were counterstained with haematoxylin.

*Immunohistochemistry for PCNA.* For the immunohistochemical detection of proliferating cells, monoclonal antibodies for PCNA (PC 10 diluted 1 : 100 in TBS) (Dako, D-2000 Hamburg, Germany) [19] were used as primary antibodies. Otherwise, the same method as described for the p53 antibody was used.

*Classification of immunohistochemistry*

Depending on the percentage of nuclei exhibiting a positive immunohistochemical staining reaction for the p53 protein, the tumours were classified into six groups: (0) tumours with a negative staining reaction; (1) < 20% positivity; (2) 20–40%; (3) 40–60%; (4) 60–80% and (5) 80–100% positivity. The immunohistochemical reaction for the p53 protein was considered to be positive only when there was nuclear staining. For analytical purposes, the highest category obtained in each patient was considered. Five separate slides per patient were reviewed and classified by two independent investigators.

The immunohistochemical reaction for the proliferation marker PCNA was classified into six groups according to the classification of the staining reaction for the p53 protein: (0) negative reaction; (1) < 20%; (2) 20–40%; (3) 40–60%; (4) 60–80% and (5) 80–100% positivity.

*Statistical calculations*

Univariate analysis using a log rank test was employed for each factor alone to determine its prognostic significance for

survival. For p53 immunohistochemistry, patients were divided into groups revealing > 20, > 40 and > 60% positivity and for each of these three cut-off levels, the correlation between positive staining and survival was calculated. Chi-squared tests with Yates corrections were used to calculate the influence of the aforementioned variables on the immunohistochemical reactivity for p53 protein. Survival was calculated from the time between surgical intervention (radical cystectomy) and death from tumour or date of last follow-up according to the Kaplan–Meier method. Finally, multivariate Cox regression analysis was used to determine whether any of the factors tested, age, sex, performance of systemic chemotherapy, tumour stage, lymph node status, presence of resectable distant metastases, histological grade or PCNA- and p53-positivity could be identified as independent prognostic factors for survival.

## RESULTS

The muscle invasive bladder tumours investigated for overexpression of the p53 protein using the monoclonal antibody 1801 were classified into 5 subgroups depending on the result of the immunohistochemical staining reaction : negative reaction, 6 patients; < 20% positivity, 11 patients; 20–40% positivity, 4 patients; 40–60% positivity, 11 patients; 60–80% positivity, 6 patients; and 80–100% positivity, 6 patients. A positive staining reaction as a predictor of survival for each subgroup alone (log rank test) achieved statistical significance at a cut-off level of ≥ 40% of cells stained positively for the p53 oncoprotein (Figure 1). Based on this calculation 21 patients (48%) were classified into Group A (<40% p53 positivity) and 23 patients (52%) into Group B (≥ 40% p53 positivity). All 5 cases of normal bladder tissue were completely negative for the p53 staining. Normal mesenchymal cells in all 44 bladder tumours did not exhibit any nuclear reactivity.

With a median follow-up of 22 months, 7 of the 21 Group A patients (33%) died from tumour progression compared to 16 of the 23 Group B patients (70%). Only 1 of the 6 patients (17%) with a completely negative staining reaction for p53 died of tumour progression. The median survival times were 24 months for Group A patients and 15 months for Group B patients ($P = 0.008$). Kaplan–Meier curves for overall survival for patients of Groups A and B are shown in Figure 2.

Patients from Groups A and B were comparable with respect to their clinical characteristics. The median age was 66 years

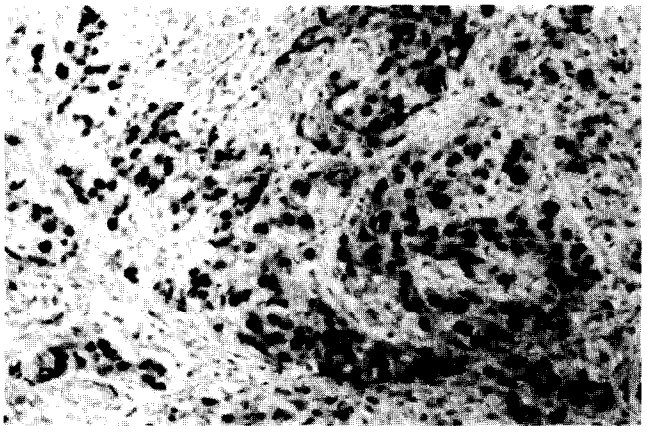

**Figure 1. Nuclear overexpression of the p53 oncoprotein in an advanced stage bladder tumour ($T_3N_2G_2$) with positive nuclear staining for the p53 oncoprotein.**

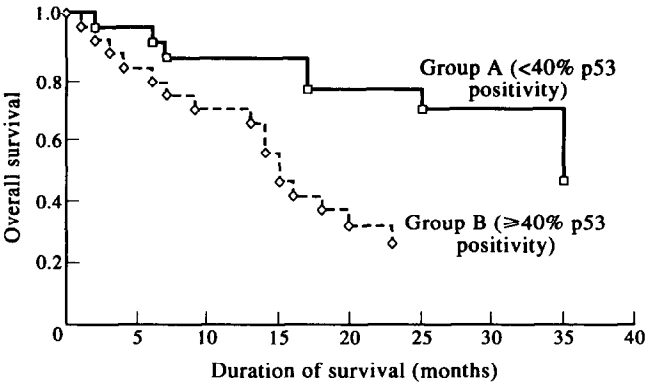


**Figure 2. Overall survival for patients in groups A and B. Group B has a significantly shorter survival time ($P = 0.008$). Kaplan–Meier survival curves.**

in both groups. 4 patients from Group A received adjuvant chemotherapy (3–4 courses of M-VAC) in comparison with 5 patients from Group B, although 2 were because of regional lymph node metastases.

In tumours from Group A patients, positive staining reactions for PCNA were observed as follows: 4 patients, < 20% positivity; 3 patients, 20–40%; 3 patients, 40–60%; 9 patients, 60–80%; 2 patients, 80–100% positivity. In 9 of the 23 (39%) tumours from Group B patients, more than 80% of cells stained positively for PCNA compared with 3 of the 21 (14%) tumours from Group A patients ($P < 0.01$).

*Statistical analysis*

Univariate statistical analysis (Table 1) demonstrated that the time of survival following radical cystectomy was independent of age ($P = 0.824$), sex ($P = 0.904$), tumour grading ($P = 0.104$), lymph node status ($P = 0.764$) and application of adjuvant systemic chemotherapy ($P = 0.542$). However, a significant correlation was found with the depth of tumour infiltration (T-stage) ($P = 0.01$), p53 positivity ($P = 0.008$) (≥ 40% of cells) and proliferative activity as indicated by PCNA positivity (≥ 80% of cells positive) ($P = 0.01$).

With multivariate analysis, T-stage ($P = 0.012$) and reactivity for the p53 oncoprotein (≥ 40% positivity) ($P = 0.023$) proved to be the only statistically relevant prognostic factors for survival as shown in Table 1.

Chi-squared tests were performed to compare p53 over-

*Table 1. Statistical analysis of prognostic factors for survival in 44 patients with muscle invasive bladder cancer*

| Factor investigated | Prognostic value (Univariate) | Prognostic value (Multivariate) |
|---|---|---|
| Sex | no (0.904) | no (0.767) |
| Age | no (0.824) | no (0.621) |
| Tumour grade | no (0.104) | no (0.952) |
| T-stage | yes (0.01) | yes (0.012) |
| Lymph node status | no (0.764) | no (0.509) |
| Chemotherapy | no (0.542) | no (0.980) |
| p53-positivity* | yes (0.008) | yes (0.023) |
| PCNA-positivity* | yes (0.01) | no (0.807) |

*p53: ≥ 40% of cells positive; PCNA: ≥ 80% of cells positive.

expression with histological grading ($P = 0.167$), age ($P = 0.783$), nodal status ($P = 0.360$), sex ($P = 0.392$) and depth of infiltration ($T_{2/3}$; $T_{3/4}$; $T_{2/4}$ tested as variables during chi-squared test, respectively). These variables were not found to be significantly correlated to the p53 protein overexpression. However, p53 overexpression was demonstrated to be significantly correlated with proliferative activity as determined by the immunohistochemical staining for PCNA ($\geq$ 80% positivity) ($P = 0.023$).

3 of the 8 patients with a muscle invasive bladder tumour of stage $T_2$ died of tumour progression, as did 8 with $T_3$ and 12 with stage $T_4$. The median survival for patients with $T_3$ and/or $T_4$ tumours was 21 months (24 months for $T_3$ and 15 months for $T_4$ tumours).

For patients in Group A and Group B, median survival was 24 months and 15 months, respectively. For $T_3/T_4$ tumours with overexpression of the p53 oncoprotein, the median survival time was 16 months [18 months for $T_3$ ($P = 0.003$) and 15 months ($P = 0.6471$) for $T_4$ tumours]. Patients with $T_3/T_4$ tumours without p53 overexpression had a median survival time of 25 months (32 months for $T_3$ and 15 months for $T_4$ tumours).

## DISCUSSION

Investigation of human malignancies for genetic alterations, including the amplification of oncogenes and the mutational inactivation of tumour suppressor genes, has resulted in an improved understanding of carcinogenesis as a multistage process, consisting of a sequence of several genetic events during malignant transformation and disease progression as, for example, demonstrated for colon cancer [20].

Approximately 80% of patients with bladder tumours are initially diagnosed with superficial disease ($T_a/T_1$ and 20–30% will relapse subsequently with muscle invasive or metastatic disease. In addition, 20% of patients will present with invasive bladder cancer at initial diagnosis.

However, since a recurrence of superficial bladder cancer does not necessarily result in tumour progression in all patients, bladder cancers with a largely variable biological potential seem to exist [1]. Recent investigations have tried to identify molecular genetic alterations which would indicate the biological potential of superficial bladder cancer, in order to distinguish patients with a high risk of progression to muscle invasive disease from those who will only develop superficial recurrences.

Cytogenetic studies and molecular genetic investigations in superficial bladder cancer revealed "loss of heterozygosity" (LOH) at # 9q as an early and relatively specific chromosomal change in bladder cancer [21]. Deletions of chromosome 9 have also been demonstrated in 67% of cases of invasive bladder cancer [22, 23]. Olumi and associates [21] reported alterations of chromosome 9 as the most common genetic event in superficial and invasive bladder tumours, suggesting the inactivation of a putative tumour suppressor gene on chromosome 9 as an important event in the development of bladder cancer [21]. Following the initiation of malignant transformation of urothelial cells, cytogenetic studies have suggested non-random changes of chromosomes # 1, 5, 7, 11 and 17 to be associated with further tumour development [24].

For superficial bladder tumours, alteration of the *TP53* gene has been demonstrated by PCR-directed RFLP (restriction fragment length polymorphism) and SSCP (single strand conformation polymorphisms) analysis, direct DNA-sequencing and immunohistochemical detection of p53 oncoprotein [10, 11]. While the prognostic value of a mutational inactivation of the *TP53* gene resulting in p53 gene protein overexpression has been demonstrated in superficial bladder cancer [12–15], it was the aim of the current study to determine whether p53 expression retains its prognostic value in patients with invasive tumours.

Sidranski and associates [25] reported the mutational inactivation of the *TP53* tumour suppressor gene in 11 of 18 (61%) patients with primary invasive bladder cancer [25]. Olumi and associates [23] reported LOH at # 17p, as detected by RFLP analysis, in more than 60% of high stage and grade bladder cancer specimens [23]. Presti and associates [26], investigating 34 bladder tumours of different stage and histological grading, confirmed a correlation between invasive behaviour and the frequency of allelic loss at the *TP53* gene locus.

Whether the detected alterations of the *TP53* locus possess a clinically prognostic role in muscle invasive or metastatic bladder cancer is currently under debate. Using an immunohistochemical approach, Lipponen [15] retrospectively investigated 212 bladder cancer biopsy specimens, obtained from superficial and muscle invasive tumours, for overexpression of the p53 oncoprotein, and correlated this to other important clinical and biological parameters. For the entire cohort of tumours, as well as for locally advanced bladder cancer alone ($T_2$–$T_4$), univariate analysis revealed a statistically significant correlation between overall survival and p53 overexpression. However, during multivariate analysis, T-stage and mitotic index were the only independent prognostic factors [15].

In 94 invasive bladder tumours (stages $T_2$–$T_4$), Esrig and associates [16] correlated overexpression of the p53 protein, grade, stage and lymph node status with the recurrence rate and overall survival of the patients. In this study, nuclear p53 overexpression was strongly associated with an increased risk of recurrence among tumours confined to the bladder ($T_2$–$T_{3a}$) and with decreased overall survival for $T_2$ tumours alone ($P = 0.023$). For this group of patients, multivariate analysis revealed p53 as the only independent prognostic factor, when compared to stage and grade of the tumours. For locally advanced tumours ($\geq T_{3b}$), a trend towards an increased recurrence rate and overall survival could be demonstrated, although this did not reach the level of statistical significance [16].

Sarkis and associates [17] investigated the prognostic impact of p53 nuclear overexpression on patients with invasive bladder cancer who were treated with neoadjuvant MVAC-chemotherapy. Compared to age, sex, palpable tumour mass, prechemotherapy T-stage, uni- or multifocal growth and histological grading, multivariate analysis identified p53 overexpression as a prognostic factor for overall survival ($P = 0.001$). The influence of p53 was detectable for each separate tumour stage, indicating that p53 retains its prognostic value for advanced stage bladder tumours [17].

In our study, the value of p53 overexpression was also an independent prognostic factor for survival in patients with invasive bladder cancer. Patients with more than, or equal to, 40% of cells positive for p53 had a median survival of 15 months compared with 24 months for patients with less than 40% of cells with p53 positivity determined by an immunohistochemical staining reaction (univariate: $P = 0.008$; multivariate: $P = 0.023$). This level of statistical significance for overall survival was only reached with a cut-off value of 40% of cells exhibiting a positive immunohistochemical reaction for p53.

During a previous study from our group in 69 patients with superficial bladder tumours, a cut-off value of 20% positivity for p53 was sufficient to demonstrate this independent prognostic role for disease progression ($P = 0.003$). After a median follow-

up of 46 months, 12 of 14 patients (86%) with more than 20% of cells positive for p53 had disease progression with muscle invasive growth compared with only 1 of 55 patients (2%) negative for p53 [13, 14]. The slightly higher cut-off level of 40% positivity used for invasive bladder cancer may indicate a lower prognostic sensitivity of p53 in advanced tumours or may be related to the process of disease progression, since invasive bladder tumours have, of course, developed from initially superficial cancers. This process may lead to an increase in the relative amount of p53 positive cells. Furthermore, while studies in superficial bladder cancer aim at disease progression as the terminal event for statistical analysis, in advanced tumours, overall survival is regarded as the appropriate event for which prognostic factors are needed.

Tumour stage has been identified as an additional prognostic parameter for survival during univariate and multivariate analysis in our study. The median survival time of 36 patients with $T_3/T_4$ tumours was 21 months. The detection of p53 overexpression seemed to add to the predictive value of the T-stage alone: for $T_3/T_4$ tumours with p53 positivity, median survival time was 16 months compared with 21 months for patients with $T_3/T_4$ tumours without p53 positivity. Particularly for $T_3$ tumours, a statistically significant difference between survival times observed for tumours with and without overexpression of the p53 oncoprotein was evident (24 versus 18 months, $P = 0.003$).

Prospective trials have to investigate the detection of p53 as an independent prognostic factor in advanced bladder cancer. The chance of gaining reliable prognostic information on the patients' clinical course at the time of diagnosis will probably also affect the decision about currently available treatment options, such as adjuvant chemotherapy, for patients with advanced bladder cancer [27–29].


1. Raghavan D, Shipley WU, Garnick MB, Russell PJ, Richie JP. Biology and management of bladder cancer. *N Engl J Med* 1990, **322**, 1129–1136.
2. Hopkins SC, Ford KS, Soloway MS. Invasive bladder cancer: support for screening. *J Urol* 1983, **130**, 61–64.
3. Kaye KW, Lange PH. Mode of presentation of invasive bladder cancer: reassessment of the problem. *J Urol* 1982, **128**, 31–33.
4. Skinner DG. Management of invasive bladder cancer: a meticulous pelvic node dissection can make a difference. *J Urol* 1982, **128**, 34–36.
5. Roehrborn CG, Sagalowsky AI, Peters PC. Long-term patient survival after cystectomy for regional metastatic transitional cell carcinoma of the bladder. *J Urol* 1991, **146**, 36–39.
6. Quinlan DC, Davidson AG, Summers CL, Warden HE, Doshi HM. Accumulation of p53 protein correlates with a poor prognosis in human lung cancer. *Cancer Res* 1992, **52**, 4828–4831.
7. Starzynska T, Bromley M, Ghoshi A, Stern PL. Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. *Br J Cancer* 1992, **66**, 558–562.
8. Thor AD, Moore DH II, Edgerton SM, *et al.* Accumulation of p53 tumor suppressor gene protein: an independent marker of prognosis in breast cancers. *J Natl Cancer Inst* 1992, **84**, 845–854.
9. Visakorpi T, Kallioniemi OP, Heikinen A, Koivula T, Isola J. Small subgroup of aggressive, highly proliferative prostatic carcinomas defined by p53 accumulation. *J Natl Cancer Inst* 1992, **84**, 883–887.
10. Dalbagni G, Saez GT, Oliva MR, *et al.* P53 mutations in bladder cancer: correlation between immunohistochemistry (IHC), single strand conformation polymorphism (SSCP) and sequencing. *J Urol* 1993, **149**, 238A.
11. Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc-70-p53 complex with an altered half life. *Mol Cell Biol* 1988, **8**, 531–539.
12. Sarkis AS, Dalbagni G, Cordon-Cardo C, *et al.* Nuclear overexpression of p53 protein in transitional cell bladder carcinoma: a marker for disease progression. *J Natl Cancer Inst* 1993, **85**, 53–59.
13. Serth J, Kuczyk MA, Bokemeyer C, *et al.* P53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the bladder. *Br J Cancer* 1995, **71**, 201–205.
14. Bokemeyer C, Kuczyk MA, Serth J. P53 – Immunohistochemistry as a prognostic marker in superficial bladder cancer. *N Engl J Med* 1994, **330**, 865.
15. Lipponen PK. Over-expression of p53 nuclear oncoprotein in transitional-cell bladder cancer and its prognostic value. *Int J Cancer* 1993, **53**, 365–370.
16. Esrig D, Elmajian D, Groshen S, *et al.* Accumulation of nuclear p53 and tumor progression in bladder cancer. *N Engl J Med* 1994, **331**, 1259–1264.
17. Sarkis A, Bajorin D, Cordon-Cardo C, *et al.* The prognostic value of p53 nuclear overexpression in invasive bladder cancer treated with neoadjuvant MVAC. *J Urol* 1994, **151**, 443A.
18. Banks L, Matlashewski G, Crawford L. Isolation of human-p53-specific monoclonal antibodies and their use in the studies of human p53 expression. *Eur J Biochem* 1986, **159**, 529–534.
19. Waasem H, Lane DP. Monoclonal antibody analysis of the proliferating cell nuclear antigen (PCNA): Structural conversation and the detection of a nucleolar form. *J Cell Sci* 1990, **90**, 121–129.
20. Nigro JM, Baker SJ, Preisinger AC. Mutations in the p53 gene occur in diverse human tumor types. *Nature* 1989, **342**, 705–708.
21. Olumi AF, Skinner EC, Tsai YC, Jones PA. Molecular analysis of human bladder cancer. *Semin Urol* 1990, **8**, 270–277.
22. Tsai YC, Nichols PW, Hiti AL, *et al.* Allelic loss of chromosome 9, 11 and 17 in human bladder cancer. *Cancer Res* 1990, **50**, 44–47.
23. Olumi AF, Tsai YC, Nichols PW, *et al.* Allelic loss of chromosome 17p distinguishes high grade from low grade transitional cell carcinomas of the bladder. *Cancer Res* 1990, **50**, 7081–7083.
24. Borland RN, Brendler CB, Isaacs WB. Molecular biology of bladder cancer. *Hematol Oncol Clin North Am* 1992, **6**, 31–39.
25. Sidranski D, Eschenbach A, Tsai YC, *et al.* Identification of p53 gene mutations in bladder cancers and urine samples. *Science* 1991, **252**, 706–709.
26. Presti JC, Reuter VE, Galan T, *et al.* Molecular genetic alterations in superficial and locally advanced human bladder cancer. *Cancer Res* 1991, **51**, 5405–5409.
27. Skinner DG, Daniels JR, Russell CA, *et al.* The role of adjuvant chemotherapy following cystectomy for invasive bladder cancer: a prospective comparative trial. *J Urol* 1991, **145**, 459.
28. Prout GR, Shipley WU, Kaufman DS. Preliminary results in invasive bladder cancer with transurethral resection, neoadjuvant chemotherapy and combined pelvic irradiation plus cisplatin chemotherapy. *J Urol* 1990, **144**, 1128–1134.
29. Logothetis CJ, Johnson DE, Chong C. Adjuvant cyclophosphamide, doxorubicin, and cisplatin chemotherapy for bladder cancer: an update. *J Clin Oncol* 1988, **6**, 1590–1596.


**Acknowledgement**—M.A. Kuczyk and J. Serth were supported by "Deutsche Krebshiefe".