TP53 Accumulation Predicts Improved Survival in Patients Resistant to Systemic Cisplatin-based Chemotherapy for Muscle-invasive Bladder Cancer


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ABSTRACT

To examine retrospectively the prognostic significance of TP53 immunoreactivity for both tumor response and patient survival in 83 patients with nonmetastatic muscle-invasive bladder cancer treated with a single transurethral resection (TUR) of tumor and combined cisplatin-based systemic chemotherapy followed by repeat TUR, paraffin-embedded sections of a bladder tumor obtained at TUR before chemotherapy (1 T2, 52 T3, and 30 T4) were immunostained for TP53 using monoclonal PAb1801 and DO-7 antibodies.

For the entire cohort, TP53 immunopositivity (PAb1801 or DO-7) did not predict complete response (CR), complete or partial response (PR), progressive disease, or time to death from bladder cancer. There was a highly significant correlation between PAb1801 and DO-7 nuclear immunoreactivity (r = 0.8242; P < 0.0001). In 76 patients in which complete clinical data were available, tumor stage (T2/T3; P = 0.0499), CR and PR (P = 0.0016) and CR (P < 0.0001) were associated with patient survival. In a multivariate model, CR (P < 0.0001) was the only independent predictor of improved survival. In complete responders, neither TP53 immunostaining nor clinicopathological factors stratified patients into prognostic groups. However, in the subset of patients (n = 38) who were chemoresistant (PR or progressive disease), improved survival was associated with ≥20% TP53 immunoreactivity (PAb1801; P = 0.0191) and tumor stage (T2/T3; P = 0.0358).

TP53 immunopositivity (PAb1801 or DO-7) did not predict overall survival or response to systemic chemotherapy in patients with nonmetastatic but predominantly clinical stage ≥T3, bladder cancer, but it had prognostic significance within the chemoresistant subgroup.

INTRODUCTION

Bladder cancer is the fourth most common malignancy in the Western male population and represents 3% of cancer deaths. In Europe, the annual incidence is 191/100,000, with a higher incidence in industrialized areas. Of new tumors, 70% are superficial (pT0/pT1), 25% are muscle-invasive (T2-T4), and 5% are carcinoma in situ types (cis). Patients with muscle-invasive disease account for the majority of deaths due to bladder cancer. In contrast, patients with superficial disease have a good prognosis, although patients with pT4G3 tumors have a 30–40% risk of progression to muscle-invasive disease (1). A number of prognostic indicators have been identified in patients with muscle-invasive bladder cancer. In a multivariate model based on clinical and pathological factors, predictors of poor cancer-specific survival are tumor stage, lymph node status, carcinoma in situ, positive surgical margins, patient’s age at surgery, and histological grade (2). A large tumor (≥5 cm) is associated with a poor outcome, stage for stage and grade for grade. Other nonspecific features of poor prognostic importance include systemic signs such as anemia, renal failure, and performance status. Patients with organ-confined disease (T1a) have a 50–60% 5-year survival, whereas those whose tumors have penetrated beyond the detrusor muscle (T2/T3a) and/or have local nodal metastases have a 15–20% 5-year survival (3). Clearly, approximately 50% of patients presenting with muscle-invasive bladder cancer have occult metastases that manifest themselves within 12 months. Few patients with systemic metastatic disease survive more than 2 years.

Early phase II studies with cisplatin in patients with metastatic bladder cancer showed response rates of 30–40% (4, 5), and therefore, the DNA damaging agent, cisplatin, has been incorporated into the majority of combination chemotherapy regimens. The two most widely used systemic regimens are M-VAC(1), which was pioneered at the Memorial Sloan-Kettering Hospital (6), and cisplatin, methotrexate, and vinblastine, which was developed by the Stanford group (7). In a landmark
The study, 246 fully evaluable patients were randomized in a prospective study to receive M-VAC or cisplatin for metastatic TCC (8). Response rates were superior for the M-VAC regimen compared with single-agent cisplatin (39% versus 12%). Overall survival (12.5 versus 8.2 months) was also significantly higher for the combined regimen.

Radical cystectomy and radical radiotherapy are, respectively, the standard surgical and bladder-preserving therapies for locally invasive bladder cancer. In these patients, the impact of neoadjuvant chemotherapy followed by cystectomy or cystectomy and adjuvant chemotherapy is still being studied. When extravesical disease (stage T3b) is suspected, there are theoretical advantages to neoadjuvant chemotherapy compared with adjuvant chemotherapy. These include better treatment tolerance, improved resectability rates, and the desirability of addressing metastatic disease at the earliest opportunity. The results of a small series of patients (n = 41) with unresectable disease treated with the M-VAC regimen followed by radical cystectomy have been reported (9). Of particular interest, the long-term benefit of subsequent radical cystectomy appeared to be confined to those patients who experienced a CR to chemotherapy.

Despite established clinicopathological prognostic indicators, none is accurate enough to identify on an individual basis those patients who would benefit from early radical therapy, neoadjuvant chemotherapy, or neoadjuvant radiotherapy, and therefore improve cancer-specific survival. The tumor suppressor gene, TP53, has been described as the most commonly mutated gene in human cancer. Highly significant associations have been reported in bladder cancer between TP53 protein immunoactivity and response to chemotherapy, which has been highly significant associations. Diffuse immunostaining for TP53 and TP53 gene mutations are detected in about 50% of muscle-invasive bladder tumors (11, 14). However, it is clear that significant accumulation of the TP53 protein can arise in the absence of detectable mutations in exons 4–9 of the TP53 gene (12, 13); therefore, TP53 status cannot be reliably detected by using current immunohistochemical methods. TP53 immunoreactivity has nevertheless been reported to be associated with poor clinical outcome in patients with muscle-invasive bladder cancer, particularly in those with organ-confined disease (15, 16). In the largest series of 243 patients treated with radical cystectomy, ≥10% TP53 immunoreactivity in TCC confined to the bladder (pT1, pT2, and pT3a) was associated with a significant risk of recurrence and death independent of tumor stage, grade, and lymph node status (15).

There is, however, controversy regarding TP53 immunoreactivity and response to chemotherapy, which has been highlighted with respect to bladder cancer (17, 18). A large body of in vitro and in vivo experimental evidence has indicated that cells with damaged TP53 are more resistant to chemotherapy because of impaired apoptosis and cell cycle checkpoint arrest, particularly with agents that damage DNA, such as cisplatin, Adriamycin, and epirubicin, which are commonly used for bladder cancer (19). In 90 patients with nonmetastatic muscle-invasive bladder cancer treated with neoadjuvant M-VAC chemotherapy and radical cystectomy, ≥20% TP53 staining using the PAb1801 antibody was independently associated with early death, and the impact on survival was predominantly in those with T2 and T3a disease (20). In 111 patients with T2-T4b N0 M0 disease treated with maximum TUR and neoadjuvant M-VAC, a total of 60 patients achieved a complete clinical response. In these patients, stratification of the primary tumor for stage and TP53 status (<20% TP53 staining) demonstrated that all patients with T2 TP53-negative tumors survived for 10 years (16 after bladder-sparring surgery and 3 after cystectomy) compared with 47% of 19 with T2 TP53-positive tumors, 67% of 12 with T3 TP53-negative tumors, and 60% of 10 with TP53-positive tumors. This suggests that bladder preservation for up to 10 years can be achieved in patients with T2 TP53-negative tumors that respond completely to neoadjuvant chemotherapy (21).

In contrast, in 32 patients again with no systemic metastases, muscle-invasive bladder cancer treated with radical cystectomy and randomized to receive adjuvant cisplatin, cyclophosphamide, and Adriamycin combination chemotherapy (22), ≥10% of TP53 staining detected using the PAb1801 antibody was reported to be associated with a 3-fold decrease in recurrence and a 2.6-fold increased chance of survival (17). To add further to the controversy, in a series of 50 patients with metastatic bladder cancer treated with M-VAC chemotherapy, TP53 immunoreactivity in the primary tumor failed to predict clinical response or survival (23).

A North of England study group assessed the benefit of combination cisplatin-based chemotherapy for nonmetastatic muscle-invasive bladder cancer (24). In the present study, we have examined the prognostic significance of TP53 immunoreactivity in this cohort of patients.

**Patients and Methods**

**Patients.** A total of 83 patients with T2-T4ab N0 M0 bladder cancer who were treated with TUR of the tumor and combination cisplatin-based chemotherapy used as initial treatment between August 1983 and January 1991 were identified (24). The paraffin-embedded archival bladder tumors that were available for TP53 immunostaining constituted the basis for this study. TUR biopsy was performed before chemotherapy, and the paraffin-embedded material was used for the immunohistochemical analysis. No attempt was made to resect the whole tumor, which remained in situ as an indicator lesion for the assessment of response to chemotherapy. Thus, the majority of the patients had residual tumor left in the bladder after the TUR. The initial tumor was staged using the Tumor-Node-Metastasis classification (UICC; Ref. 25). For histological grading, the WHO system was used (26). All patients underwent extensive radiological assessment to exclude occult nodal spread and distant metastasis, which included a computed tomography scan of the abdomen and pelvis and chest radiography. Skeletal isotope scans were performed for patients with bone pain. No patient had evidence of lymph node metastasis or distant metastasis at the initiation of chemotherapy.

**Treatment.** Four different cisplatin-based chemotherapy regimens were used (Table 1). The regimen of 43/83 patients assessed included two of three DNA-damaging agents (cisplatin, Adriamycin, and its derivative epirubicin). Our series essentially represented a combination of chemotherapy regimens undergo-
Table 1  Patients treated with the different cisplatin-based chemotherapy regimens for the entire cohort (n = 83) and those patients assessed and stratified according to response to chemotherapy (n = 71)

<table>
<thead>
<tr>
<th>Type of chemotherapy</th>
<th>Chemo-sensitive subgroup (n = 33)</th>
<th>Chemoresistant subgroup (n = 38)</th>
<th>No. of patients in entire cohort (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin and methotrexate</td>
<td>10</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Cisplatin, methotrexate, and vinblastine</td>
<td>10</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Cisplatin, methotrexate, and epirubicin</td>
<td>10</td>
<td>20</td>
<td>37</td>
</tr>
<tr>
<td>M-VAC</td>
<td>3</td>
<td>3</td>
<td>6</td>
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</tbody>
</table>

Table 2  Details of adjuvant salvage treatments for patients with muscle-invasive bladder cancer that are either chemosensitive or chemoresistant treated with TUR of tumor and combination chemotherapy

<table>
<thead>
<tr>
<th>Salvage treatments</th>
<th>Chemosensitive subgroup (n = 33)</th>
<th>Chemoresistant subgroup (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radical radiotherapy</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Palliative radiotherapy</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Radical radiotherapy and subsequent radical cystectomy</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Radical cystectomy</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Partial cystectomy</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>27</td>
</tr>
</tbody>
</table>

During phase II trials. All patients in the study group were scheduled to receive a total of six cycles of systemic chemotherapy, although upon evidence of nephrotoxicity, either this was reduced or chemotherapy was abandoned. The study investigating the effect of TUR of the tumor and combination cisplatin-based chemotherapy in patients with nonmetastatic bladder cancer was approved by the local ethics committee. All patients gave written or verbal informed consent for the study and were aware of the results of phase II trials. Upon the detection of disease progression or distant nodal/metastatic spread, patients were reassessed and treated with salvage therapies. This included radical or partial cystectomy or radical or palliative radiotherapy. A few patients had a combination of the two treatment modalities. The options for salvage treatment for recurrent disease were based on appropriate clinical decisions made by the patient’s urologist (Table 2).

All patients underwent a check cystoscopy and examination under anesthesia within 1 month of completion of their chemotherapy. Thorough TUR of the site of the tumors was performed, and the tissue obtained was sent for pathological assessment.

The pathological response to chemotherapy assessed at the first check cystoscopy was divided into three categories: (a) CR: The absence of tumor in the bladder with no evidence of distant nodal or visceral spread; (b) PR: Down-staging of the tumor to a superficial stage (cist/T1) or decrease in the bulk of the initial invasive tumor by 50%, with no evidence of distant or nodal spread; and (c) PD: Persistent invasive disease not downsized by chemotherapy, with or without evidence of nodal or distant spread.

Throughout the follow-up period, patients’ responses were assessed clinically and by regular cystoscopies and TUR of the tumor. Median follow-up for survivors and those last seen alive was 78 (range, 1–121) months.

Immunohistochemical Staining for TP53. Four-micrometer paraffin-embedded sections of formalin-fixed bladder cancer were cut and picked up on glass slides coated in 2% 3-aminopropyltriethoxysilane solution in acetone. The sections were then de-waxed in xylene and hydrated through graded alcohols. Heat-mediated antigen retrieval was performed using a sodium citrate buffer (pH 6.0) in a microwave processor (Energy beam Sciences H2500) for 20 min at 95°C. After the microwave heating, the sections were left to stand for a further 20 min in hot buffer. After rinsing in running water, endogenous peroxidase was quenched using 3% hydrogen peroxide for 10 min. After treatment with PBS (pH 7.1), other nonspecific activity was reduced by incubation in normal goat serum (diluted 1:5 in PBS) for a further 10 min. The sections were incubated overnight at 4°C in the following dilutions of primary TP53 monoclonal antibodies: (a) PAb1801 (Novocastra Laboratories, code NCL-p53–1801) diluted 1:150 in PBS; and (b) DO-7 (DAKO UK Ltd., code M7001) diluted 1:250 in PBS.

After washing in PBS, immunostaining of tumor specimens was continued with the streptavidin-biotin technique using the Biotin-Streptavidin Amplified detection systems (B-SA systems) with horseradish peroxidase label (Biogenex) kit. Sections were incubated in biotinylated multilink antibody (Biogenex) laboratories, code NCL-p53–1801) diluted 1:150 in PBS; and (b) DO-7 (DAKO UK Ltd., code M7001) diluted 1:250 in PBS.

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Assessment of Immunostaining. Scoring was performed separately by two independent observers (K. N. Q and T. R. L. G) and recorded blind in relation to the clinical information. The entire area of the tumor was assessed, and only areas of the tumor that had stained intensely were analyzed. One thousand tumor nuclei were counted commencing in regions, with the most positively stained tumor nuclei and the percentage positivity recorded. The mean scores for percentage of immunoreactivity were calculated for the two observers if the initial difference was ≤10%. If the discrepancy was >10%, both observers would rescore the slides together and come to an agreement regarding the final score.

Statistical Methods. The following statistical analyses were performed using Minitab for Windows (Release 9.2) software. Data are presented as medians with ranges. Grouped data were compared using the Mann-Whitney U test for two non-parametric distributions. Proportions were compared using Fisher’s exact test. Reproducibility of staining and comparisons of results expressed as percentage of positivity were assessed using Spearman’s rank correlation coefficient (r). This was performed using SPSS (Release 6.1) software. Two-tailed P values of ≤0.05 were considered statistically significant.

Various thresholds of TP53 immunopositivity using DO-7 antibody influencing time to death due to TCC or treatment-related death were analyzed by plotting Kaplan Meier curves using Prism (Release 2.01) software (27), whereas for PAb1801, an established cutoff of ≥20% TP53 staining was used (14, 20, 23). The survival probabilities as a function of time were compared using Log-rank analysis. Patients who died of causes other than TCC were considered as censored data. Patients who died of unknown causes were excluded from the analysis. Categorical parameters influencing survival were compared using Cox’s proportional hazards regression (28). Factors that demonstrated statistical significance in the univariate model were then included in the multivariate model using a forward conditional stepwise procedure to identify variables of independent significance. Crude and adjusted relative risks were also calculated. This was performed using SPSS (Release 6.1) software.

RESULTS

The median age of the 83 patients included in the study was 66 (range, 47–84) years. Fifty-nine were male, and 24 were females. One patient presented with a T3 tumor, 52 had T3 tumors, and 30 had T2 bladder tumors. The majority of tumors were TCCs, although a minority possessed squamous or adenomatous differentiation. Nine patients had grade II tumors, and 74 patients had grade III tumors. The 5-year survival for the entire cohort was 42%, and for patients with T2/T3 disease, it was 52%. Clinical response was assessed in 94% (78/83) of patients in the study group. CR occurred in 45% (35/78), PR occurred in 17% (13/78), and PD developed in 39% (30/78) of patients, whereas the CR rate for T2/T3 tumors was 55%. Patients that had a CR to chemotherapy had significantly improved survival (71% at 5 years) when compared to patients that had a PR (17% at 5 years) or developed PD (22% at 5 years; P < 0.0001, log-rank test; Fig. 1). With regard to the distinct prognostic groups uncovered in relation to response to chemotherapy, those patients that demonstrated a CR were deemed chemosensitive, and those that had a PR or PD were designated as chemoresistant. During the follow-up period, 52 patients (63%) required adjuvant salvage treatment because of recurrent disease after TUR of tumor and combination chemotherapy. There was no significant difference in survival between those patients treated solely with TUR of the tumor and combination chemotherapy and patients who developed subsequent disease progression and required adjuvant salvage treatment (P = 0.58, Log-rank test). There was also no difference between the frequency and type of salvage treatments subsequently given in patients deemed chemosensitive or chemoresistant (P = 0.12, χ² test; Table 2) or in the number of cycles of chemotherapy administered to these patients. Similarly, there was no significant difference in response (P = 0.29, χ² test) or cancer-specific survival when stratifying for the various chemotherapy regimens used (Table 1).

Immunoreactivity was assessed using the two antibodies, PAb1801 and DO-7, as described. Immunoreactivity was only observed in tumor cells (Fig. 2), and an absence of staining was demonstrated in normal bladder tissue in the samples analyzed which acted as an internal control. Scores from the two observers for the PAb1801 antibody that differed by >10% amounted to 17 of the 83 (21%) sections, whereas that for DO-7 was 24%. The Spearman correlation for the paired scores for PAb1801 and DO-7 was r = 0.8699 (P < 0.0001) and r = 0.9004 (P < 0.0001), respectively. The interantibody correlation for the final mean scores for the nuclear immunoreactivity detected by PAb1801 and DO-7 was r = 0.8242 (P < 0.0001).

There was no significant difference in nuclear immunoreactivity between groups of patients that had been stratified according to clinical response, i.e., CR (chemosensitive), PR and PD (chemoresistant), and PD for either of the two antibodies used (CR versus PD, P = 0.467 (PAb1801) and P = 0.854 (DO-7), Mann Whitney U test; CR versus PR/PD, P = 0.787
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B

Table 3 shows the results of the univariate and multivariate Cox regression analysis, the relative risk, and the probability of various prognostic indicators in predicting cancer-specific survival for the entire cohort of patients treated with TUR of the tumor then subsequent combination chemotherapy. A total of 76 patients in whom complete survival data were available were included in this model. In the univariate analysis of T2/T3 tumors (P = 0.0499), CR and PR (P = 0.0016), and CR (P < 0.0001) were predictors of improved survival. For the entire cohort, patients with tumors expressing ≥20% PAb1801 and ≥10% (DO-7) nuclear immunoreactivity for TP53 were not significantly associated with survival at the 95% confidence level (P = 0.15 and P = 0.14, respectively, Log-rank test using categorization; Fig. 3). Similarly, TP53 immunoreactivity analyzed as a continuous variable in the univariate model was not associated with significance. In the multivariate model, CR (P < 0.0001) was the only independent predictor for improved survival for this group of patients.

When the whole study group was analyzed with regards to response at first check cystoscopy, a different relationship was discovered for patients who demonstrated a CR (n = 33) and thus were chemosensitive and those that were chemoresistant (n = 38). Table 4 shows the results of the univariate Cox regression analysis for predictors of survival in patients that were either chemosensitive/resistant after treatment with TUR of tumor and combination chemotherapy. There was no significant difference in patients that were chemosensitive with regards to cancer-specific survival when stratifying for clinicopathological factors or TP53 staining. The absence of a relationship for the mentioned variables was also found for patients with T2/T3 tumors. However, for patients that were chemoresistant (noncomplete-responders), T2/T3 tumors (P = 0.0358) and ≥20% TP53 (PAb1801) immunoreactivity (P = 0.0191) were the only predictors of improved survival. Patients with tumors demonstrating ≥20% TP53 immunoreactivity with PAb1801 staining (n = 11) were associated with significantly improved survival (P = 0.0119, log-rank test), with a 5-year survival of 50%; for those with <20% TP53 immunoreactivity (n = 27), the 5-year survival was 8% (Fig. 4). Similarly, those patients that were chemoresistant and survived >5 years, 75% (6/8) demonstrated ≥20% TP53 immunopositivity, whereas only 18% (7/39) were TP53-positive and died (P = 0.0033, Fisher’s exact test).

DISCUSSION

In a series of patients with muscle-invasive nonmetastatic bladder cancer containing 82/83 patients (99%) clinically staged with ≥T3 disease, we have found that TP53 immunoreactivity assessed at diagnostic TUR using PAb1801 or DO-7 antibodies does not predict response to subsequent combination systemic cisplatin-based chemotherapy. Absence of a correlation between TP53 staining of the primary tumor and response to M-VAC chemotherapy has previously been reported for patients with nonmetastatic (20) and metastatic disease (23). In our series, the CR rate for the entire cohort was 45% and for patients with T2/T3 tumors, 55%. This compares favorably with a recent series in which 54% of patients with T2-T3 N0 M0 disease showed a CR after combination M-VAC chemotherapy (29). Our comparable CR rate for T2/T3 tumors was achieved despite only one patient possessing a T2 tumor and only 6/83 (7%) patients having received the M-VAC regimen. This suggests that the combined cisplatin-based chemotherapy regimens used in our study have similar efficacy when compared to the M-VAC regimen with regards to CR. Our results do not support a prospective study assessing the value of TP53 staining in patients who were initially treated with radical cystectomy and randomized to adjuvant chemotherapy (cisplatin, cyclophosphamide, and Adriamycin; Ref. 17). In contrast to our results, these authors showed increased sensitivity to chemotherapy in patients with TP53 immunopositive tumors. Their findings were consistent with the mechanisms of chemosensitivity suggested in cell lines (30), although the prevailing view from most in vitro and preclinical in vivo models indicate that mutant TP53 is associated with resistance to chemotherapy (19). The original randomized prospective study evaluating the impact of adjuvant chemotherapy (22) has been criticized (31). A substantial proportion of patients who were candidates for entry were not entered into the study, perhaps indicating a degree of selection.

(PAb1801) and P = 0.944 (DO-7), Mann Whitney U test]. Nuclear immunoreactivity was significantly higher with DO-7 than with PAb1801 staining when comparing results for the whole study group (P = 0.003, Mann-Whitney U test).

Fig. 2 Photomicrograph of an invasive bladder tumor stained with (A) PAb1801 and (B) DO-7 antibodies.
This debate is clearly important, but the discrepancy may have arisen because at present, TP53 immunohistochemistry alone cannot determine whether the TP53 pathway is intact. This requires assessment of other aspects of the TP53, including TP53 gene sequencing, cell cycling, apoptosis, p21 levels, or MDM2 status. Clearly this needs evaluation with regard to chemosensitivity.

Cancer-specific survival in the entire cohort of patients or restricted to those with organ-confined disease could not be predicted on the basis of TP53 staining. This contrasts with previous retrospective series in which ≥20% TP53 immunopositivity (PAb1801) was an independent predictor of reduced overall survival in patients treated with neoadjuvant chemotherapy and early radical cystectomy (20) or neoadjuvant radiotherapy and early radical cystectomy (32). In the neoadjuvant chemotherapy series, the impact on survival was greatest in patients with organ-confined tumors (T2/T3a), whereas in the latter series, the prognostic value rested with stage T3b patients.

Although not evaluated in a randomized prospective trial, a bladder-sparing approach has been proposed as an alternative strategy in patients who achieve a CR to chemotherapy. In a series of 43 patients who had bladder-sparing surgery after a CR to neoadjuvant M-VAC chemotherapy, the 10-year survival rate was 74%, which included 58% with an intact functioning bladder (29). In our series, the 5-year survival in complete responders was 71%, which included 82% with an intact bladder. These excellent results were achieved despite only six (7%) patients having the M-VAC regimen.

In patients with T2-T4 disease who undergo a bladder-sparing strategy, we are unable to predict, on the basis of existing clinicopathological and molecular markers, survival in those that are complete (chemosensitive) and noncomplete (chemoresistant) responders. We therefore assessed TP53 immunoreactivity as a predictor of patient survival in these subgroups. TP53 staining or clinicopathological parameters did not stratify patients that were chemosensitive into good or poor prognostic groups in terms of cancer-specific survival. This is in contrast to a recent study that stratified 60 patients with T2/T3 N0 M0 disease that achieved a clinical CR to M-VAC by TP53 status; those tumors with <20% TP53 staining possessed a survival advantage compared to those tumors that had ≥20% TP53 staining (21). However, in patients who were chemoresistant, univariate analysis revealed that ≥20% TP53 staining (PAb1801) and T2/T3 disease were significant predictors of prolonged survival (Table 4; Fig. 4). Unlike chemosensitive patients in which bladder-sparing is a reasonable option in many patients, most chemoresistant patients are expected to undergo radical treatment. Therefore, knowing the TP53 status in chemoresistant patients is unlikely to alter clinical management, although it does provide valuable prognostic information.

### Table 3

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variables (numbers in parenthesis)</th>
<th>Univariate model</th>
<th>Multivariate model</th>
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<tr>
<td>Tumor size</td>
<td>&lt;5 cm (31), ≥5 cm (27)</td>
<td>(0.2713)</td>
<td>(0.6862)</td>
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<td>TP53 IR (PAb1801)</td>
<td>Absolute values</td>
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<tr>
<td>TP53 IR (DO-7)</td>
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<td>(0.1561)</td>
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<td>≥20% (21), &lt;20% (55)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Tumor stage</td>
<td>T3, T4 (41)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>CR and PR</td>
<td>Yes (45), No (26)</td>
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<td>CR</td>
<td>Yes (33), No (38)</td>
<td>&lt;0.0001</td>
<td>5.36 (2.51–11.48)</td>
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</table>

*RR, relative risk; CI, confidence interval.

Not significant.

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In patients with T2-T4 disease who undergo a bladder-sparing strategy, we are unable to predict, on the basis of existing clinicopathological and molecular markers, survival in those that are complete (chemosensitive) and noncomplete (chemoresistant) responders. We therefore assessed TP53 immunoreactivity as a predictor of patient survival in these subgroups. TP53 staining or clinicopathological parameters did not stratify patients that were chemosensitive into good or poor prognostic groups in terms of cancer-specific survival. This is in contrast to a recent study that stratified 60 patients with T2/T3 N0 M0 disease that achieved a clinical CR to M-VAC by TP53 status; those tumors with <20% TP53 staining possessed a survival advantage compared to those tumors that had ≥20% TP53 staining (21). However, in patients who were chemoresistant, univariate analysis revealed that ≥20% TP53 staining (PAb1801) and T2/T3 disease were significant predictors of prolonged survival (Table 4; Fig. 4). Unlike chemosensitive patients in which bladder-sparing is a reasonable option in many patients, most chemoresistant patients are expected to undergo radical treatment. Therefore, knowing the TP53 status in chemoresistant patients is unlikely to alter clinical management, although it does provide valuable prognostic information.

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**Fig. 3** Cancer-specific survival for the entire cohort stratified according to TP53 immunoreactivity [P = 0.15, Log-rank test (PAb1801)]. TP53+ [≥20% IR]; TP53− [<20% IR].
Table 4  Univariate Cox regression analysis of risk factors in predicting cancer-specific survival in patients with muscle-invasive bladder cancer treated by TUR of tumor and combination chemotherapy that are chemosensitive (n = 33) and those that are chemoresistant (n = 38)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Variables</th>
<th>Chemoresistant tumors</th>
<th>P</th>
<th>Crude RR* (95% CI)</th>
<th>Chemosensitive tumors</th>
<th>P</th>
<th>Crude RR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53 IR (PAb1801)</td>
<td>Absolute values</td>
<td>(0.2072)*</td>
<td>1.0</td>
<td>(0.2636)*</td>
<td>(0.5594)*</td>
<td>1.0</td>
<td>(0.09–1.03)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td>(0.3370)*</td>
<td>1.0</td>
<td>(0.5900)*</td>
<td>(0.6696)*</td>
<td>1.0</td>
<td>(0.38–6.07)</td>
</tr>
<tr>
<td>Tumor stage</td>
<td>T2/T3/T4</td>
<td>(0.0358)</td>
<td>1.0</td>
<td>(0.74)</td>
<td>(0.73)</td>
<td>1.0</td>
<td>(0.18–2.93)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>&lt;5 cm</td>
<td>(0.0191)</td>
<td>3.23</td>
<td>(0.74)</td>
<td>(0.74)</td>
<td>0.74</td>
<td>(0.18–2.96)</td>
</tr>
<tr>
<td>Tumor size</td>
<td>≥5 cm</td>
<td>(0.0191)</td>
<td>3.23</td>
<td>(0.74)</td>
<td>(0.74)</td>
<td>0.74</td>
<td>(0.18–2.96)</td>
</tr>
</tbody>
</table>

* RR, relative risk; CI, confidence interval.

In conclusion, assessment of TP53 immunoreactivity does not predict response to cisplatin-based chemotherapy and fails to predict overall cancer-specific survival in our series containing all but one patient clinically staged with \( \geq T_3 \) nonmetastatic bladder cancer. TP53 staining does not stratify patients who are chemosensitive into different prognostic groups. However, in patients who are chemoresistant, \( \geq 20\% \) TP53 staining (PAb1801) is associated with improved survival, and this should be confirmed in extended prospective multicenter studies.

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