Allelic imbalance at 1p36 may predict prognosis of chemoradiation therapy for bladder preservation in patients with invasive bladder cancer

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Invasive bladder cancers have been treated by irradiation combined with cis-platinum (CDDP) as a bladder preservative option. The aim of this study was to find a marker for predicting patient outcome as well as clinical response after chemoradiation therapy (CRT) by investigating allelic loss of apoptosis-related genes. A total of 67 transitional cell carcinomas of the bladder treated by CRT (median dose: 32.4 Gy of radiation and 232 mg of CDDP) were studied. We investigated allelic imbalances at 14 loci on chromosomes 17p13 and 1p36 including the p53 and p73 gene regions by fluorescent multiplex PCR based on DNA from paraffin-embedded tumour specimens and peripheral blood. The response to CRT was clinical response (CR) in 21 patients (31%), partial response (PR) in 31 (46%), and no change (NC) in 15 (22%). There was no statistical correlation between treatment response and clinical parameters, such as tumour grade, stage, radiation dose, or CDDP dose. The frequencies of allelic imbalance for TP53 and TP73 were 21.4 and 36.8%, respectively; neither was correlated with clinical treatment response and tumour stage or grade. There was no statistical correlation between treatment response and allelic imbalance at the other 12 loci. We found a significant correlation between cancer-specific survival and an imbalance of D1S243 (P = 0.0482) or TP73 (P = 0.0013) using a Log-rank test, although other loci including TP53 did not correlate with survival (P = 0.4529). Multivariate analysis showed performance status (P = 0.0047), recurrence (P = 0.0017), and radiation doses (P = 0.0468) were independent predictive factors for cancer-specific survival. However, an allelic imbalance of TP73 was the most remarkable independent predictive factor of poor patient survival (P = 0.0002, risk ratio: 3382). Our results suggest that the allelic loss of the p73 gene predicts a clinical outcome of locally advanced bladder cancer when treated by CRT.

Initial treatments for invasive bladder cancer can be divided into bladder-sparing therapy and radical cystectomy. Although radical cystectomy remains the standard treatment, a variety of adjuvant therapies have been attempted to induce tumour downstaging and to improve survival. During the last three decades, the most commonly used bladder-sparing treatment has been external beam radiation therapy (Shipley et al., 1987). Studies of systemic chemotherapy combined with radiation prior to surgery have shown that it can allow bladder preservation by transurethral resection (TUR) or partial cystectomy in 52–70% of patients with invasive bladder cancer (Tester et al., 1993; Tester et al., 1996; Kachnic et al., 1997; Shipley et al., 1997; Shipley et al., 1998). We have treated locally advanced bladder cancer by a modified version of Shipley’s regimen using radiation combined with cis-platinum (CDDP) (Shipley et al., 1987). Although many tumours show a good response to this treatment, several are resistant. Since this therapy can be unnecessarily harmful for patients whose tumours do not respond, if we could predict the response of a bladder cancer to this treatment, it would help choose appropriate candidates for this therapy regime.

Dysfunction of the p53 tumour suppressor gene located at 17p13.1 is the most frequent alteration in human malignancy and frequent loss of heterozygosity (LOH) on 17p13.3, distal to p53, has been reported in breast, colon, ovary, and lung cancers (Tsuchiya et al., 2000). Mutant p53 protein overexpression is frequently observed in bladder cancers that show more malignant behaviour. Loss of p53 function is one of the most important roles in resistance to chemotherapy or radiation-induced apoptosis. The incidence of LOH of chromosome 17p or mutation of p53 is 60% or more in advanced bladder cancer (Sidransky et al., 1991). However, more than a few patients with bladder cancer harbour p53 alterations and do respond to CDDP. This finding suggests that there are some pathways other than p53 activation that mediate CDDP-induced cell death, and that damage to these pathways leads to clinical crisis.

The p73 gene that has been mapped to chromosome 1p36.3, which has a high homology to the p53 gene, is also a key factor in the apoptotic pathway. It has been reported that p73 is a
component of a mismatch repair-dependent pathway, and that p73 levels are increased after DNA damage (Agami et al., 1999; Gong et al., 1999; Yuan et al., 1999). Many studies have suggested that there may be several tumour suppressor genes on 1p36, such as in neuroblastoma and hepatocellular carcinoma (Ichimiya et al., 1999; Fang et al., 2000).

This evidence prompted us to investigate specific loci encompassing p53 and p73 genes by microsatellite analysis using the fluorescent multiplex PCR technique, and to investigate genetic markers that could help predict the clinical outcome of chemoradiotherapy in bladder cancer.

**MATERIALS AND METHODS**

**Patients**

A cohort of 67 patients, who underwent preoperative chemoradiotherapy (CRT) for locally invasive (T2-4N0M0) or high-risk superficial (pT1G3) bladder cancer between November 1994 and August 2000, was studied (Table 1). Before treatment, we performed cold cup biopsies on tumours and selected-site bladder biopsies that were taken from trigone, both lateral walls, verumontanum, dome, anterior wall, bladder neck, and prostatic urethra in men. Systemic work-up for tumour staging on CT scanning, intravenous pyelography, and bone scintigram were performed for all patients. Tumours were graded histopathologically according to the WHO classification and were staged by the TNM staging system of the UICC (1992). All patients received preoperative chemotherapy with cis-diamino-dichloro-platinum (CDDP) intravenously plus radiotherapy. The regimen (based on Shipley's method) was CDDP (70 mg/m²) on day 1, with radiation administered by LiniaC to the true pelvis at 1.8 Gy/day per fraction from day 2 to day 6 in the first week, and 5 consecutive days per week thereafter. The therapy was carried out one to three times every 14 days. The mean total dose was 40.5 Gy, ranging from 30 to 61.2. At the completion of one cycle of CRT, patients were treated by CDDP of 70 mg/m² and irradiation of 16.2 Gy to the basic target. Although we tried to perform three cycles of CRT when possible, in those patients who received one or two cycles of the therapy and who had persistent side effects such as nausea, vomiting, diarrhoea, and pancytopenia for 2 weeks or who refused to continue with CRT, the treatment was halted. At 4 weeks after the completion of CRT, patients were assessed for response by selected-site mucosal cold cup biopsy or TUR and CT scanning. The clinical response to CRT was classified as follows: CR (clinical response), pathologically no tumour residual in the bladder (pCR) and no evidence of nodal or visceral metastasis; PR (partial response), downsizing of the tumour with a decrease of over 50% compared to the initial tumour, with no evidence of distant or nodal spread on CT scanning. Cases that were pathologically not pCR were classified as PR even if the tumour showed complete disappearance radiographically. NC (no change): persistent invasive disease that was not downsized by CRT, with or without evidence of nodal or distant spread. Patients whose tumour showed NC or whose residual tumour cells remained in the muscle layer were referred for radical or partial cystectomy with lymph node dissection, while patients with responding tumours (CR or PR) underwent complete resection of the cancer by TUR. Six patients who had just residual carcinoma *in situ* (CIS) were subsequently treated by intravesical instillation of Mitomycin C (MMC) or BCG. Treatment-related toxicities were evaluated by WHO criteria. Cystoscopic examination followed by washing cytology was carried out every 3 months from the third to fifth year and every 6 months thereafter. Complementary examinations, including chest X-rays and/or CT scans, were carried out every 6 months.

**Specimens and DNA extraction**

Specimens of tumours collected at the time of initial diagnosis were studied for the source of tumour DNA. Formalin-fixed paraffin-embedded tissues were cut into 5-μm sections, and the first and last sections were used for histopathological analysis with HE stain. Specimens containing at least more than 75% tumour cells were used for analysis. Specimens from three-step sections were used for DNA extraction using DEXPAT (Takara, Tokyo, Japan) according to the manufacturer’s instructions.

**Blood collection**

Blood samples from each patient were collected as a normal control. Blood was treated with EDTA, and lymphocyte DNA was isolated using a GenTLE extraction kit (Takara, Tokyo, Japan).

**PCR amplification**

Totally, 14 microsatellite markers containing loci on chromosome 17p13 and 1p36, with two- or three-base pair repeat motifs were investigated. The primer sequences for these markers were obtained from the Genome Database (http://gdbwww.gdb.org). The location of each marker is shown in Table 2. Polymerase chain reaction (PCR) was carried out in a reaction volume of 10 μl containing 0.2 μl of each primer (one of the primers was labelled with FAM, HEX, or TET), 2 μl MgCl₂, 0.2 μl dNTP, 1 U of Taq polymerase, and approximately 5 ng of genomic DNA. Amplification was carried out by 40 PCR cycles of 94°C for 45 s, 55°C for 60 s, and 72°C for 45 s.

**Multiplex PCR**

The multiplex PCR method was the same as described above, with the exception that 2–5 primer pairs were added in the same PCR tube. Primers were selected on the basis that the resultant PCR products could be distinguished by colour and size without overlapping.

**Polyacrylamide gel electrophoresis**

The PCR products were subjected to electrophoresis on 4% polyacrylamide denaturing gel in TBE buffer and analysed using an...
Table 2 Prevalence of allelic imbalance, and observed heterozygosities at different loci in lp and 17p

| Marker | Locus  | No/Informative case (%) | Observed heterozygosities |
|--------|--------|-------------------------|--------------------------|
| 1 D1S171 | lp36.3 | 19/55 (34.5)            | 0.78                     |
| 2 TP73  | lp36.2–36.3 | 32/57 (56.1)       | 0.86                     |
| 3 D1S243 | lp36.2–36.3 | 18/57 (32.1)       | 0.86                     |
| 4 D1S244 | lp36.2–36.3 | 19/57 (32.8)       | 0.86                     |
| 5 D1S507 | Ip36.1–36.2 | 20/57 (35.1)       | 0.83                     |
| 6 D1S547 | Ip36.3–36.1 | 24/57 (42.1)       | 0.81                     |
| 7 8ABR  | lp3.3  | 28/57 (49.1)            | 0.96                     |
| 8 D17S1574 | lp3.3 | 15/56 (26.8)          | 0.68                     |
| 9 D17S520 | Ip3.3 | 19/54 (35.2)          | 0.63                     |
| 10 D17S1566 | lp3–13.3 | 12/57 (21.1)       | 0.72                     |
| 11 D17S201 | Ip3–13.3 | 18/55 (32.7)       | 0.89                     |
| 12 ENO3  | Ip3.2  | 19/55 (32.7)            | 0.89                     |
| 13 TP53  | lp3.1  | 12/57 (21.1)            | 0.72                     |
| 14 D17S520 | lp2–13.1 | 15/58 (25.9)       | 0.74                     |

automated fluorescent DNA sequencer (model 377, Applied Biosystems, CA, USA). In total, 1 μl of each PCR product was resuspended using 3.5 μl of loading solution (2.5 μl formamide, 0.5 μl of 50 mm EDTA with 50 mg ml⁻¹ Blue Dextran, and 0.5 μl of Gene Scan Size Standard: GS500). This mixture was denatured at 95 °C for 5 min and a 1.5 μl aliquot of the mixture was used for analysis. Electrophoresis was performed for 2 h at 40 W and 52 °C.

Data analysis and calculation of allele ratios

To detect allelic imbalance, the peak area of the two alleles was calculated by Gene Scan Analysis Software for each tumour specimen and the corresponding lymphocyte DNA. Allelic imbalance was defined as a decrease of the signal of one allele by more than 50% in tumour DNA when compared with lymphocyte DNA.

Statistical analysis

Correlations of frequency of allelic imbalance with tumour grade, stage, and response to CRT were assessed using χ² or Fisher’s exact tests. Survival after CRT was analysed using the Kaplan–Meier method with a Log-rank test as univariate analysis. Multivariate analysis was performed by using Cox’s proportional hazard regression test in step-wise mode. Data were processed using JMP 4.0 statistical software, with P<0.05 indicating statistical significance.

Ethical considerations

Our research was carried out in accordance with the principles set out in the Declaration of Helsinki 1964 and all subsequent revisions, informed consent was obtained, and the ethical review committee of Yamaguchi University School of Medicine approved the study.

RESULTS

Treatment response and prognosis

A total of 67 patients (median age: 68 years; range: 45–89) received CRT with a median follow-up period of 32.6 months (range: 2.9–84.2) and were eligible for evaluation (Table 1). The average total dose of CDDP and radiation was 230 mg (median 220 mg, range: 75–375) and 40.5 Gy (median 32.4, range: 30–61.2), respectively. Treatment-related toxicity such as anemia, leucopeny, thrombocytenia, nausea, vomiting, and diarrhoea were mild and tolerable. Severe toxicity over grade 3 was not observed. The response to CRT was CR in 21 patients (31%), PR in 31 (46%), and NC in 15 (22%). Recurrence was noted in 26 patients (39%), including 11 with progressive disease. Of the patients who achieved pCR, nine (42.8%) had recurrence. Three patients had superficial local recurrence, and six had invasive local recurrence or distant metastases. A total of 15 patients suffered cancer-related death.

No statistical relation was found when treatment response (CR or non-CR) was compared with tumour stage, grade CDDP doses, or radiation doses (Table 4).

Deletion mapping of chromosome 1 and chromosome 17

Table 2 shows the prevalence of allelic imbalance and the fraction of heterozygosity, which ranged between 0.68 and 0.96. Figure 1 summarises the results of allelic imbalance of the 14 microsatellite markers. Not done was defined either as when DNA amplification failed for technical reasons, or when the specific chromosomal allele did not show heterozygosity with the control lymphocyte DNA. Of the 67 tumours, allelic imbalance was observed from 21.1 to 56.1% in 14 markers. A significantly higher frequency of allelic imbalance was observed in the p73 locus than those at the p53 locus (56.1 vs 21.1%, P = 0.003).

Relation of allelic imbalance to clinicopathological parameters and treatment response

Table 3 shows the relationship of allelic imbalance to tumour stage and grade. Except for the D1S507 locus, no statistically significant association was found between allelic imbalance and tumour stage or grade at any locus. At the D1S507 locus, allelic imbalances were observed at a significantly high frequency in the progressive stage (P<0.0305). As for the relationship between allelic imbalance and treatment response, significant differences of allelic imbalance were not observed between CR and non-CR patients at each locus (Table 4).

Correlation between cause-specific survival and allelic imbalance

Table 5 shows the correlation between cause-specific survival and candidate-predictive factors including allelic imbalance at each locus. Significant correlation was observed between allelic imbalances of TP73 (imbalance: death 10 vs alive 22; balance: death 0 vs alive 25, P = 0.0013) or its neighbour locus, D1S243 (imbalance: death 6 vs alive 12; balance: death 4 vs alive 34, P = 0.0482) and survival rate; whereas no association was observed at TP53 (imbalance: death 3 vs alive 9; balance: death 8 vs alive 37, P = 0.4529). Clinical parameter, grade, stage treatment response, CR or non-CR (imbalance: death 4 vs alive 17; balance: death 13 vs alive 33, P = 0.04916), and CDDP dose did not correlate with cause-specific survival, although significant correlation was observed between survival rate and recurrence or no recurrence (P = 0.0027), performance status (0.0113), and radiation doses (P = 0.0486), respectively. By multivariate analysis before treatment, an allelic imbalance of TP73 was the most useful independent predictive factor in predicting cause-specific survival in bladder cancer patients treated by CRT (Table 5, P = 0.0002, Risk ratio: 3382). Figure 2 plots the Kaplan–Meier’s survival curves stratified by treatment response, and allelic imbalance of TP73, D1S243, and TP53.
Figure 1 Deletion mapping on chromosomes 17p13 (A) and 1p36 (B) in bladder cancer. Hetero, heterozygote; Homo, homozygote; AI, allelic imbalance; RER, replication error.
treatments before chemotherapy; thus, having a much worse prognosis than the group of patients in our study. Sengelov’s series might have had more heterogeneous changes of genes than that of our series. In our study, patients were only locally advanced primary treated cases and not recurrent or metastatic cases. Therefore, we believe that their series would not have shown a significant correlation between allelic imbalances and response to chemotherapy or duration of survival. LOH at 17p is common, 40–63%, in patients with advanced bladder cancer (Olumi et al., 1990; Tsai et al., 1990; Dalbagni et al., 1993; Reznikoff et al., 1996). Many tumours with LOH at the 17p locus have been shown to have mutations in the retained 17p allele (at the p53 locus) affecting mutated P53 protein overexpression. p53 loss is one of the most important events in the tumour suppressor or regulator cell cycle and apoptosis. In addition, expression of p53 is induced by several types of DNA damage caused by CDDP or irradiation. LOH at 17p (including the p53 locus) have been shown to predict a poor response to chemotherapy in head and neck cancer. In contrast to these data, our results are in good agreement with another study showing a lack of relation between p53 mutation and chemotherapy response (Sengelov et al., 2000).

Another region of interest is on 17p13.3, telomeric to the TP53 locus in several types of tumours, where the presence of new tumour suppressor genes is indicated in hepatocellular carcinoma, malignant astrocytoma, paediatric primitive neuroectodermal tumours, breast cancer, high grades of astrocytic tumours, and ovarian cancer (Biegel et al., 1992; Saxena et al., 1992; Nishida et al., 1993; Ishimura et al., 1994; Phillips et al., 1996; Chattopadhyay et al., 1997). In lung cancer, new tumour suppressor gene(s) may reside on three distinctly deleted regions on chromosome 17p13.3 distal to the p53 gene, with possible roles in progression and differentiation of adenocarcinomas (Tsuchiya et al., 2000). Thus, we expected that new tumour suppressors or apoptosis-related genes may reside in 17p13 regions and investigated LOH of the TP53 locus and its distal regions. However, we did not find any correlation between the prevalence of LOH at each locus and tumour grade, stage, or response to CRT. In our study, the prevalence of LOH at the TP53 locus was not very frequent. These results suggest that LOH study at the p53 gene and its neighbour locus is not important for predicting chemoradio-sensitivity or survival in locally advanced bladder cancer. However, loss of function of p53 by gene deletion or mutation is a more important factor in the malignant potential of bladder cancer, and further examination is needed to predict the clinical course treated by CRT.

The distal region of the short arm of human chromosome 1 (1p36) is thought to contain tumour suppressor genes, such as p73 and RIZ, in a variety of cancers. The p73 gene, a member of p53 gene family, is also an important gene reflecting apoptosis or tumorigenesis in bladder cancer. The p73 gene has been mapped at chromosome 1p36.3, a region frequently deleted in breast cancer, neuroblastoma, and other malignancies (Han et al., 1999; Ichimiyata et al., 1999; Ahomadegbe et al., 2000). RIZ, retinoblastoma protein (Rb)-interacting zinc-finger gene (PRDM2), was isolated during functional screening for Rb-binding proteins (Buyse et al., 1995) and was mapped to 1p36.2, the RIZ locus is a target of frequent deletion in hepatocellular carcinoma (Fang et al., 2000). Several reports have suggested that p73 is not likely to be a tumour suppressor gene, and that its overexpression by the activation of a silent allele may contribute to the progression of bladder cancer (Chi et al., 1999; Yokomizo et al., 1999). In contrast, Ono et al. (2001) demonstrated that the absence of CDDP-induced apoptosis in p53-defective TCC cells, established CDDP-resistant sublines from the TCC cell line T24, may be attributable to a loss of p73 induction that would be followed by insufficient caspase activation. Our results are partially consistent with their data, leading to the hypothesis that inactivation of p73 may be the cause of acquired resistance to CDDP observed in TCC in clinical settings.

**DISCUSSION**

We applied a newly developed fluorescent multiplex-PCR technique, which achieves superior resolution when compared with isotopic methods, to microsatellite detection. We found loss of heterozygosity (LOH) at many loci on chromosomes 1 and 17 in 67 bladder cancers. Previous studies have identified LOH at 1p, 4p, 4q, 6p, 8p, 9p, 9q, 10q, 11p, 13q, 14q, 17p, and 18q in advanced bladder cancers (Tsa et al., 1990; Reznikoff et al., 1996). Close association of LOH found at specific locations to prognosis as well as chemotherapy response was reported in other malignancies (Feugeas et al., 1996). Sengelov et al. (2000) reportedly found no predictive markers including LOH at 1p, 8p, 10p, 13q, and 17p for response to chemotherapy in advanced bladder cancer. Their data is in contrast with our data showing the close association of allelic imbalance at p73 with cause-specific survival in patients who were treated by CRT in bladder cancer, although the prevalence of allelic imbalance at p73 did not correlate with treatment response. We consider that their series analysed patients with more advanced disease (metastatic or recurrent) and with resistant to various treatments before chemotherapy; thus, having a much worse prognosis than the group of patients in our study. Sengelov’s series might have had more heterogeneous changes of genes than that of our series. In our study, patients were only locally advanced primary treated cases and not recurrent or metastatic cases. Therefore, we believe that their series would not have shown a significant correlation between allelic imbalances and response to chemotherapy or duration of survival.

**Table 3**

| Variable | CR | Non-CR | P-value |
|----------|----|--------|---------|
| Microsatellite markers | | | |
| 1 D1S171 | 7.3 | 27.3 (%) | 0.1489 NS |
| 2 TP73 | 16.7 | 40.7 | 0.6953 |
| 3 D1S243 | 12.5 | 19.6 | 0.5895 |
| 4 D1S244 | 8.9 | 23.2 | 0.7633 NS |
| 5 RIZ | 16.1 | 23.2 | 0.3748 |
| 6 D1S2057 | 12.3 | 22.8 | 0.9191 |
| 7 D1S1566 | 8.8 | 33.3 | 0.0878 NS |
| 8 ABR | 12.3 | 33.3 | 0.1879 |
| 9 D1S1574 | 8.9 | 19.7 | 1.0000 |
| 10 YNZ22 | 9.3 | 24.1 | 0.7635 NS |
| 11 D1S1566 | 5.3 | 15.8 | 0.7338 |
| 12 ENO3 | 9.1 | 25.2 | 0.5442 |
| 13 TP53 | 8.9 | 23.2 | 0.7633 |
| 14 D1S520 | 6.9 | 19.0 | 0.7515 |
| Clinical parameters | | | |
| G2 or G3 | 31.3 | 68.7 (%) | 0.7601 |
| p53, p73, and RIZ | 31.3 | 68.7 | 0.3066 NS |
| CDDP dose* | 216 | 237 (mg) | 0.2107 |
| Radiation dose* | 40.9 | 40.5 (Gy) | 0.8665 |

*Mean dose. CR = clinical response; CDDP = cis-platinum; NS = not significant.

**Table 4**

| Variable | G2/G3(%) | T1/T2/T3 & T4 (%) | P-value |
|----------|---------|--------------------|---------|
| Histological grade | | | |
| 1 D1S171 | 9.1/25.5 | 0.4845 NS | |
| 2 TP73 | 13.0/44.4 | 0.5014 NS | |
| 3 D1S243 | 5.4/26.8 | 1.0000 NS | |
| 4 D1S244 | 5.4/26.8 | 0.7320 NS | |
| 5 RIZ | 5.4/33.9 | 0.3287 NS | |
| 6 D1S257 | 7.0/28.1 | 0.0000 NS | |
| 7 D1S247 | 8.8/33.3 | 0.0000 NS | |
| 8 ABR | 12.3/36.8 | 0.6982 NS | |
| 9 D1S1574 | 7.1/19.6 | 0.8345 NS | |
| 10 YNZ22 | 7.4/25.9 | 0.4740 NS | |
| 11 D1S520 | 7.0/17.5 | 0.7008 NS | |

**NS** = not significant.

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It is likely that p73 overexpression may occur during an early to intermediate phase of bladder cancer. Cancer cells at that time acquire a more malignant behaviour, and they could lose one allele at many loci, including the p73 gene, due to genomic instability at an advanced stage of bladder cancer. Indeed, overexpression of p73 gene may be unnecessary for cancer cell survival in a late phase.

In conclusion, our results suggest that the allelic imbalance at p73 may be a useful prognostic indicator in bladder cancer patients treated by CRT. This may enable appropriate candidates for CRT.

Table 5  Univariate and multivariate analyses for predicting cause-specific survival in bladder cancer patients treated with chemoradiotherapy

| Variable Category | (a) Univariate analysis P-value | Risk ratio (95% CI) P-value |
|------------------|--------------------------------|---------------------------|
| DIS171 Balance or imbalance | 0.2588 NS | 3382 (not calculated) 0.0002 |
| TP73 | 0.0013 NS |
| D1S243 | 0.0482 NS |
| D1S244 | 0.8444 NS |
| RIZ | 0.6178 NS |
| D1S507 | 0.2756 NS |
| D1S247 | 0.9640 NS |
| ABR | 0.2051 NS |
| D1S1574 | 0.1859 NS |
| YNZ22 | 0.3886 NS |
| D1S1566 | 0.5149 NS |
| ENO3 | 0.7176 NS |
| TP53 | 0.4529 NS |
| D1S520 | 0.2632 NS |
| Microsatellite markers Age ≤ 68 years or > 68 years | 0.6652 NS |
| PS 0 or >0 | 0.0113 NS |
| Tumour grade G2 or G3 | 1.0000 NS |
| Tumour stage T1 or T2 or T3 & T4 | 0.5989 NS |
| Recurrence Yes or No | 0.0027 NS |
| Response CR or non-CR | 0.4916 NS |
| CDDP doses ≤ 220 mg or > 220 mg | 0.3634 NS |
| Radiation doses ≤ 32.4 Gy or > 32.4 Gy | 0.0486 1.655 (1.007–2.935) 0.0468 |
| Clinical parameters | | |
| PS 0 or >0 | 0.0113 NS |
| Tumour grade G2 or G3 | 1.0000 NS |
| Tumour stage T1 or T2 or T3 & T4 | 0.5989 NS |
| Recurrence Yes or No | 0.0027 NS |
| Response CR or non-CR | 0.4916 NS |
| CDDP doses ≤ 220 mg or > 220 mg | 0.3634 NS |
| Radiation doses ≤ 32.4 Gy or > 32.4 Gy | 0.0486 1.655 (1.007–2.935) 0.0468 |

*Used Log-rank test. †Used the Cox proportional hazard test. CDDP = cis-platinum; CR = clinical response; NS = not significant.

Figure 2  Kaplan–Meier survival curves stratified by TP53 (A), TP73 (B), DIS243 (C), and treatment response (D).

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to be selected from among patients with locally advanced bladder cancer. Patients with an allelic imbalance at p73 may be considered for immediate cystectomy, rather than for bladder preservation by CRT. Investigation of a larger number of patients with additional follow-up treatment by CRT is required to validate our preliminary results.

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