**Table 1.** General characteristics of patients with castration resistant prostate cancer treated with docetaxel

| Variable | Category | Number of Patients: All n = 80 (%) | CRP Status: Nonelevated (<5 mg/L) n = 34 (%) | CRP Status: Elevated (≥5 mg/L) n = 46 (%) | P |
|---|---|---|---|---|---|
| Age (y) | <70 | 41 (51) | 16 (47) | 25 (54) | .651 |
| | ≥70 | 39 (49) | 18 (53) | 21 (46) | |
| ECOG PS | 0 | 66 (83) | 32 (94) | 34 (74) | .034 |
| | ≥1 | 14 (17) | 2 (6) | 12 (26) | |
| Gleason score | <8 | 14 (17) | 6 (18) | 8 (17) | .981 |
| | ≥8 | 66 (83) | 28 (82) | 38 (83) | |
| Analgesic consumption | −ve | 40 (50) | 23 (68) | 17 (37) | .012 |
| | +ve | 40 (50) | 11 (32) | 29 (63) | |
| Bone metastasis | −ve | 10 (12) | 10 (29) | 0 (0) | <.001 |
| | +ve | 70 (88) | 24 (71) | 46 (100) | |
| Visceral metastasis | −ve | 57 (71) | 23 (68) | 34 (74) | .621 |
| | +ve | 23 (29) | 11 (32) | 12 (26) | |
| Prior estrogen | −ve | 7 (9) | 4 (12) | 3 (7) | .451 |
| | +ve | 73 (91) | 30 (88) | 43 (93) | |
| PSA (ng/mL) | <80 | 48 (60) | 25 (74) | 23 (50) | .040 |
| | ≥80 | 32 (40) | 9 (26) | 23 (50) | |
| Albumin (g/dL) | ≥3.6 | 52 (65) | 27 (79) | 25 (54) | .032 |
| | <3.6 | 28 (35) | 7 (21) | 21 (46) | |
| ALP (IU/l) | <450 | 42 (53) | 24 (71) | 18 (39) | .007 |
| | ≥450 | 38 (47) | 10 (29) | 28 (61) | |
| Hemoglobin (g/dL) | ≥11.0 | 44 (55) | 24 (71) | 20 (43) | .023 |
| | <11.0 | 36 (45) | 10 (29) | 26 (57) | |

NS = not significant.

2010 and comprised the current study cohort. In general, patients received 75 mg/m$^2$ of docetaxel intravenously every 3 weeks. If necessary, dose reduction and/or interval extension was allowed, based on a patient's overall condition. The median number of docetaxel chemotherapy cycles was 6 (range 1-23). Corticosteroid was simultaneously administered to all patients. In addition, zoledronic acid was administered to 30 patients (38%) with bone metastases. Seventy-three patients (91%) had already been given estrogen before docetaxel therapy was initiated. Forty patients (50%) had been using analgesics, including morphine, for pain control before the docetaxel therapy. All patients provided written, informed consent.

### Variables

Prognostic variables were as follows: age at the beginning day of first cycle on docetaxel chemotherapy, ECOG PS, Gleason score, presence or absence of analgesic consumption, bone metastasis, visceral metastasis and prior estrogen therapy, pretreatment levels of serum prostate-specific antigen (PSA), hemoglobin, albumin, ALP, and CRP. For the statistical analysis, the categories of age, ECOG PS, and Gleason score were subdivided into 2 groups (age <70 years vs ≥70 years, ECOG PS 0 vs ≥1, and Gleason score 6-7 vs 8-10). The cut-off points of PSA, hemoglobin, albumin, ALP, and CRP were set at 80 ng/mL, 11.0 g/dL, 3.6 g/dL, 450 IU/L, and 5 mg/L, with the highest value of "sensitivity − (1 − specificity)" in the receiver operating characteristics (ROC) analysis using overall death as an endpoint, respectively.

### Statistical Analysis

Because the primary endpoint of this study was overall survival, the follow-up period was defined as the initial day of the first cycle of docetaxel chemotherapy to the date of death or last visit. The associations among clinicopathological features were analyzed using the Fisher's exact test. The Kaplan–Meier curves were used to determine overall survival rate. The differences in overall survival rates were assessed using the log-rank test. Prognostic variables for overall survival were evaluated using the Cox proportional hazard model using backward elimination. For entry into a multivariable model, the $P$ value of bivariate results was set to .25. The concordance index (c-index) was calculated as reported elsewhere.[17] For all analyses, the differences were considered significant at $P$ <.05. All statistical analyses were performed using JMP software version 5.0 (SAS Institute, Inc., Cary, NC).

## RESULTS

During the follow-up period (median 9.4 months, range 1-31 months), 37 of the 80 patients (46%) died of prostate cancer and 1 (1%) of another cause. The median survival period of all patients was 14.5 months (95% CI 10.7-21.8).

Characteristics of all 80 patients are summarized in Table 1. The median baseline CRP level was 6 mg/L (interquartile range 2-14 mg/L). CRP status was significantly associated with ECOG PS, presence or absence of analgesic consumption and bone metastasis, and pretreatment levels of serum PSA, hemoglobin, albumin, and ALP.

Bivariate and multivariable analyses for overall survival in patients with CRPC treated with docetaxel were shown in Table 2. The bivariate analysis revealed that ECOG PS, ALP, hemoglobin, albumin, PSA, and CRP were associated with overall survival. In the multivariable analysis using backward elimination, both CRP and hemoglobin were independent prognostic factors for overall survival. The hazard ratio of hemoglobin and

**Table 2.** Bivariate and multivariable analyses for overall survival in patients with CRPC treated with docetaxel

| Variable | Category | Bivariate *P* | Multivariable: Full Model *P* | Multivariable: Final Model: Regression Coefficient | Multivariable: Final Model: HR (95% CI) | Multivariable: Final Model: *P* |
|---|---|---|---|---|---|---|
| Age (y) | <70 vs ≥70 | .867 | | | | |
| ECOG PS | 0 vs ≥1 | .002 | .138 | | | |
| Gleason score | <8 vs ≥8 | .404 | | | | |
| Analgesic consumption | −ve vs +ve | .117 | .936 | | | |
| Bone metastasis | −ve vs +ve | .125 | .301 | | | |
| Visceral metastasis | −ve vs +ve | .277 | | | | |
| Prior estrogen | −ve vs +ve | .299 | | | | |
| PSA (ng/mL) | <80 vs ≥80 | .010 | .994 | | | |
| Albumin (g/dL) | ≥3.6 vs <3.6 | <.001 | .089 | | | |
| ALP (IU/l) | <450 vs ≥450 | .005 | .091 | | | |
| Hemoglobin (g/dL) | ≥11.0 vs <11.0 | <.001 | .089 | 0.486 | 1.63 (1.17-2.30) | .004 |
| CRP (mg/L) (continuously) | | .003 | | | | |
| CRP (mg/L) | <5 vs ≥5 | <.001 | .017 | 0.666 | 1.95 (1.33-2.96) | <.001 |

HR = hazard ratio; CI = confidence interval; NS = not significant.

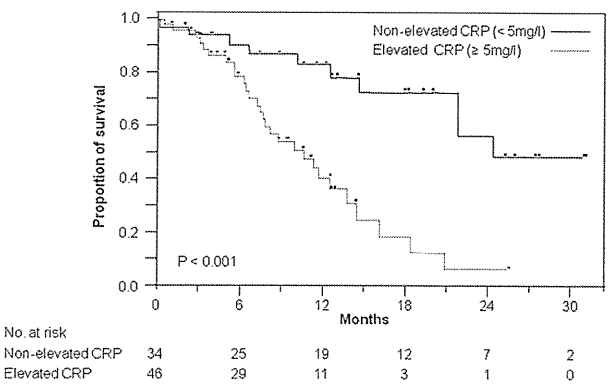


**Figure 1.** Overall survival curves for CRPC patients treated with docetaxel divided into nonelevated CRP (<5 mg/L) and elevated CRP (≥5 mg/L) groups.

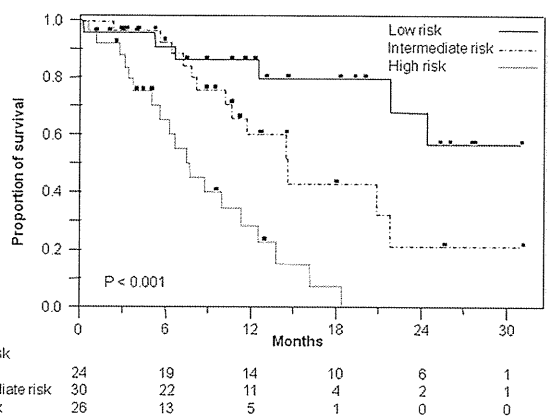


**Figure 2.** Overall survival curves for all patients with CRPC treated with docetaxel according to the risk groups. According to the presence of an elevated CRP (≥5 mg/L) concentration and/or a low hemoglobin level (<11.0 g/dL), each patient was assigned to low-, intermediate-, and high-risk groups.

CRP were 1.63 (95% CI 1.17-2.30, $P = .004$) and 1.95 (95% CI 1.33-2.96, $P < .001$), respectively. Median survival periods were 25 months in the nonelevated CRP (<5 mg/L) group and 11 months in the elevated CRP (≥5 mg/L) group ($P < .001$), respectively (Fig. 1).

Because CRP and hemoglobin were found to be 2 prognostic factors for overall survival, patients were divided into 3 risk groups according to CRP and hemoglobin levels. Both the elevation of CRP concentration (≥5 mg/L) and a low hemoglobin level (<11.0 g/dL) were assigned weight 1 because the regression coefficients of CRP and hemoglobin in the final multivariable model were nearly equivalent. According to the sum of their scores, patients were classified as low (0), intermediate (1), or high (2) risk. Overall survival curves according to the risk stratification were clearly tiered and statistically significant ($P < .001$), with 1-year survival rates of 86.3% (95% CI 56.8-95.8), 60.5% (95% CI 21.6-96.7), and 23.0% (95% CI 15.3-96.1) for low-, intermediate-, and high-risk groups, respectively (Fig. 2). Median survival periods of the patients with low-, intermediate-, and high-risk groups were not calculable, 15 months (95% CI 10-22) and 8 months (95% CI 5-11), respectively. The hazard ratio of intermediate and high risk to low risk were 1.66 (95% CI 1.04-2.83) and 3.03 (95% CI 1.90-5.21). The c-index of the risk stratification containing CRP and hemoglobin was 0.55 compared with those of 0.41 with hemoglobin alone and 0.40 with CRP alone.

## COMMENT

In the present study, we demonstrated that an elevated CRP concentration and a low hemoglobin level are significant prognostic factors for overall survival in patients with CRPC treated with docetaxel. The elevation of CRP concentration and a low hemoglobin level were associated with poor patient survival. Risk stratification containing CRP and hemoglobin can be useful for estimating the length of overall survival of these patients.

As shown by the prognostic value of CRP in various advanced cancers,[9-12,18] the presence of a systematic in-

flammatory response as evidenced by an elevation of CRP concentration could be associated with a poor outcome in patients with CRPC treated with docetaxel. Granted, 2 reports have previously reported the prognostic value of CRP in patients with CRPC.[15,16] In the present study, we expanded upon the findings of those previous reports by describing risk stratification containing both CRP and hemoglobin.

Low hemoglobin level is one of the common conditions and is also an independent prognostic factor for survival in patients with various cancers.[19] In prostate cancer, a low hemoglobin level is associated with shorter overall survival.[20] In our study, a low hemoglobin level was also shown to be an independent prognostic factor for CRPC patients treated with docetaxel.

Systemic inflammatory response is caused by the stimulation of inflammatory cytokines. Among them, interleukin-6 (IL-6) is the potent inducer of CRP production and inversely correlated with hemoglobin level.[21-23] IL-6 regulates prostate cancer cell growth in vitro[24,25] and the prostate cancer cell itself also produces IL-6 in the process of bone metastasis.[26,27] Thus, the underlying inflammatory process could stimulate prostate cancer progression in an autocrine or paracrine manner. CRP, which is a representative acute-phase reactant, could reflect the aggressiveness of prostate cancer.

In the present study, we identified prognostic factors using only prechemotherapeutic factors in a single-institute cohort. In many prognostic models in patients with CRPC, postchemotherapeutic factors, namely, PSA decline, tumor response, and pain response, are often included to evaluate prognostic factors,[7,8] but it might be more beneficial for patients to predict the response or outcome before the initiation of treatment. The current results suggest that CRPC patients treated with docetaxel could be evaluated for risk of mortality using prechemotherapeutic factors, such as CRP and hemoglobin, which is already easily measured by standardized assays in most institutions.

Neither Gleason score nor PSA were associated with overall survival length in the present study. Previous reports demonstrated that a high Gleason score ($\geq$8) could be a negative prognostic factor for overall survival. Because the cut point of Gleason score using 6-7 vs 8-10 was common, we used this cut point in the current study. However, 83% of patients had high Gleason scores of 8 or more in the current cohort and it was unlikely that Gleason score could have been found to be significant because of power. Then we also considered the cut point using Gleason scores 6-8 vs 9-10, but the statistical significance was not found in bivariate analysis ($P$ = .404). In the issue, we could not observe that Gleason score was prognostic in our current cohort. This might be a result of the small sample size. PSA is the important biomarker associated with disease status of prostate cancer. Indeed, PSA is a significant prognostic factor for overall survival in the bivariate analysis. However, PSA concentrations were significantly associated with both CRP concentrations ($P$ = .040) and hemoglobin levels ($P$ = .042) in the present study. Therefore, PSA might not have impact on overall survival compared with CRP and hemoglobin in the multivariable analysis.

There are a few limitations to this study. Given the small sample size, the retrospective nature and the data from a single institution of this study, additional larger confirmatory studies are warranted to validate our results. Because CRP and hemoglobin were evaluated as dichotomous variables with cut-off points, there might be possibilities of type-1 error or overfitting in this small cohort. Because CRP is a nonspecific inflammatory marker, we should also verify that the CRP value has not changed because of other diseases or conditions in which CRP might be elevated. Despite these concerns, CRP could still function as a useful and widely available biomarker.

## CONCLUSIONS

We have identified that CRP, as well as hemoglobin, is an independent prognostic factor for overall survival of patients with CRPC treated with docetaxel. Risk stratification based on CRP and hemoglobin could be helpful for estimating the overall survival.

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Int J Clin Oncol
DOI 10.1007/s10147-011-0275-6

ORIGINAL ARTICLE

# Outcome, clinical prognostic factors and genetic predictors of adverse reactions of intermittent combination chemotherapy with docetaxel, estramustine phosphate and carboplatin for castration-resistant prostate cancer

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Received: 1 April 2011 / Accepted: 9 June 2011


**Abstract**
*Objectives* Docetaxel-based chemotherapy is effective in patients with castration-resistant prostate cancer (CRPC). This phase II study assessed the outcome and predictive factors for prognosis and toxicity following intermittent chemotherapy with docetaxel, estramustine phosphate, and carboplatin (DEC) in patients with CRPC.
*Methods* Thirty-five patients were treated with a DEC regimen that consisted of a 28-day cycle of drugs as follows: docetaxel (60 mg/m$^2$ on day 1), carboplatin (AUC 5 on day 1) and estramustine phosphate (560 mg daily). Treatment was continued intermittently. The end point was to test the effect of DEC on the response rate and overall survival (OS). Statistical correlations between the outcomes and predictive factors, including clinical parameters and 8 single-nucleotide polymorphisms (SNPs) related to drug metabolism, were assessed.
*Results* Prostate-specific antigen levels decreased by more than 30% in 65.7% of the patients. The median OS following DEC was 17.8 months, and the median total time of chemotherapy holiday was 7.7 months (range 1.7–35.8). On multivariate analysis, serum lactate dehydrogenase (LDH) was an independent prognostic factor for OS ($p = 0.007$). On SNP analysis, patients carrying the TT genotype of the *ABCB1* C3435T polymorphism showed a significantly more severe leukocytopenia during the first cycle of DEC therapy compared to patients with the CC + CT genotype ($p = 0.036$).
*Conclusion* Combination chemotherapy with DEC has a potential effect on CRPC with acceptable toxicity. Serum LDH may be a promising predictor of prognosis, and the *ABCB1* C3435T polymorphism may be a genetic predictor of the severity of leukocytopenia in patients with CRPC treated with DEC.

**Keywords** Chemotherapy · Prostate cancer · Single nucleotide polymorphism · Docetaxel

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## Introduction

Prostate cancer is currently the most common malignancy in men and the second or third leading cause of death in the Western world [1]. Although prostate cancer may be initially responsive to androgen ablation therapy, it can finally become refractory to hormonal manipulation. Two large phase III randomised trials showed that docetaxel-based chemotherapy enhanced survival in the treatment of castration-resistant prostate cancer (CRPC) [2, 3]. Furthermore, many combination therapies carry expectations of demonstrating the additive and synergistic effects of taxanes in patients with CRPC. To date, several studies have evaluated the benefit of combination chemotherapy with taxanes, estramustine phosphate (EMP) and carboplatin (TEC), of which the combination utilizing docetaxel as the taxane (docetaxel, EMP, carboplatin = DEC) was reported to have achieved particularly high response rates (58–98%) in patients with CRPC [4]. With regard to the schedule for the administration of docetaxel for CRPC, intermittent

Published online: 25 June 2011

therapy has been conducted to reduce the side effects and maintain a high quality of life (QOL) [5].

Although taxane-based chemotherapy is promising for the treatment of CRPC, it remains open to debate what type of patients will have higher survival benefit. In addition, individual variation in response to chemotherapy may result in differences in the severity of adverse reactions (ARs). This individual variation has been partially explained by single nucleotide polymorphisms (SNPs). We previously reported that SNPs in drug metabolism-related genes were associated with prognosis and ARs in urological cancer [6, 7].

Here, we report the results of a phase II study evaluating the efficacy and patient tolerance of intermittent combination chemotherapy with DEC in patients with CRPC. We also evaluated the prognostic factors and predictors of ARs, including clinical parameters and SNPs in patients with CRPC.

## Patients and methods

### Patients

Our study included patients with CRPC treated at Akita University Hospital, Japan. A history of surgical or medical castration was required in all patients. Patients were diagnosed by biochemical and/or clinical progression following either (1) a second or subsequent cycle of hormonal therapy, or (2) other regimens including monotherapies of docetaxel, EMP, dexamethasone and prednisolone. The definition of diagnosis of CRPC and its progression were based on the criteria of the Prostate Cancer Clinical Trials Working Group [8].

### Treatment regimen

The DEC regimen consisted of a 28-day cycle of docetaxel [60 mg/m$^2$ intravenously (IV) on day 1], carboplatin (IV to the area under the curve of 5 on day 1) and EMP (560 mg orally daily). Pre-medication consisting of dexamethasone (8 mg IV) was administered 30 min prior to each docetaxel infusion. Two consecutive DEC cycles were performed and efficacy and toxicity were assessed. Before further therapy with DEC, a chemotherapy holiday was taken from the treatment until the prostate-specific antigen (PSA) levels were elevated above the baseline. Dose-down regimens were prepared for elderly patients and for those with a history of severe ARs. Luteinzing hormone releasing hormone agonist was continued throughout the study. Treatment was stopped for any of the following reasons: progression of disease, severe adverse events, or withdrawal of consent.

### Evaluation

A post-therapy change in PSA levels was defined on the basis of the maximum degree of change from baseline within 3 months of therapy. Tumour progression was defined by following RECIST guidelines. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 4.0 (NCI-CTC v4.0). Levels of pre-treatment platelet count, hemoglobin, serum alkaline phosphatase (ALP), serum lactate dehydrogenase (LDH) and PSA were measured prior to initial DEC therapy, and only PSA levels were measured every 3 months thereafter. The presence of bodily pain prior to initial DEC therapy was evaluated from the patient's medical records. The primary endpoint of the study was response rate. The secondary endpoints included safety and overall survival (OS) from the time of initiation of chemotherapy. For the evaluation of prognosis, the variables which were significant predictors in the univariate analysis were included in the multivariate analysis.

### DNA extraction and genotyping

DNA was extracted from blood samples using a QIAamp Blood kit (Qiagen, Hilden, Germany). Patients were genotyped for polymorphisms of 8 SNPs in 6 genes (*MAP4*, *MAPT*, *ABCG2*, *CYP3A5*, *XRCC1* and *ABCB1*) considered to be involved in the metabolism of DEC (Table 1). The genotype of each SNP was determined either by polymerase chain reaction restriction fragment length polymorphism or by direct sequencing. Written informed consent for enrolment in this study and for the use of DNA and clinical information was obtained from all patients participating in this study, which was was approved by the institutional review board of Akita University School of Medicine. We examined the influence of age and genetic factors on OS and the development of severe toxicity during the first cycle of DEC therapy using univariate and multivariate analyses. Severe leukocytopenia was defined as greater than grade 3.

### Statistical analysis

The Kaplan–Meier method was used to estimate OS. Each continuous independent variable was dichotomised at the median value. Differences in survival were tested using the log-rank test. Hazard ratios and 95% confidence intervals (Cis) for cancer death were assessed using the Cox proportional hazard regression model. Odds ratios and 95% CIs for dichotomised grades of ARs in each genotype grade were determined by multiple logistic regression analysis. Statistical analysis was performed using SPSS 15.0®. Differences were considered significant at $p < 0.05$.

**Table 1** List of gene polymorphisms assessed in this study

| Genes | Type and site | No. | Primers | | Restriction enzyme |
|---|---|---|---|---|---|
| | | | Forward | Reverse | |
| *MAP4* | Intron | rs56313601 | TGCATGGTTTCCTTTCCCCTA | TCTCTGAAACGTGTGTGGCTT | *Bcc*I |
| *MAPT* | Intron | rs3744460 | AAAGTGGAGGCGTCCTTGCGA | CAGCTTCTTATTAATTATCTGC | *Mnl*I |
| *ABCG2* | V12M | rs2231137 | GCTTTTCTGTCTGCAGAAAGAT | GAAGCTGTCGCGGGGAAGCC | *Tsp*RI |
| *CYP3A5* | A6986G | rs776746 | ATGGAGAGTGGCATAGGAGATA | TGTGGTCCAAACAGGGAAGAAATA | *Ssp*I |
| *XRCC1* | C194T | rs1799782 | ATGCTTGGCCAGTTCCGTGTGAAG | CACCTGGGGATGTCTTGTTGATCC | *Alu*I |
| *XRCC1* | A399G | rs25487 | TCCTCCACCTTGTGCTTTCT | AGTAGTCTGCTGGCTCTGGG | *Nci*I |
| *ABCB1* | C3435T | rs1045642 | TTGATGGCAAAGAAATAAAGC | CTTACATTAGGCAGTGACTCG | *Mbo*I |
| *ABCB1* | Intron | rs7779562 | TGTTCTGCAATGAGAAGAATAA | ATTGTAACACAAATTAATTATC | *Taq*I |

## Results

### Patient characteristics

A total of 35 patients with CRPC were enrolled in the study between 2003 and 2009. Their pre-treatment characteristics are summarised in Table 2. The median age was 68 years (range 54–79). The Eastern Cooperative Oncology Group performance status (ECOG-PS) was 0–1 in 97.1% of patients. Sixteen (47.5%) patients suffered recurrence after local definitive treatments. Prior combined androgen blockade was performed in 33 (94.3%) patients, and 30 (85.7%) patients were previously administered EMP. All patients were docetaxel-naïve in this study. Under pathological examination, the grade of cancer in the majority (80.0%) of the patients was classified as 'poorly differentiated' according to the general rule for clinical and pathological studies on prostate cancer from the Japanese Urological Association and the Japanese Society of Pathology [9]. Gleason's score was 7–8 in 13 patients, 9–10 in 6 and unclassified in 16. Bone metastasis was present in 30 (85.7%) patients, while extra-osseous metastasis was present in 18 (51.4%). Median baseline PSA level at initiation of DEC regimen was 99.6 ng/mL (range 0.036–4900). Median duration from diagnosis of CRPC to initiation of DEC regimen was 14.9 months (range 1.0–109.1).

### Clinical outcomes

A median of 3 cycles (range 1–9) was administered to each patient. Clinical responses to DEC therapy are summarised in Table 3. PSA levels were decreased by >30% in 65.7% and by >50% in 45.7% of patients as a maximum response. Measurable tumours were assessed in 34.3% (12/35) patients, the response rate being 66.7% (8/12). The median follow-up time was 11.1 months (range 2.6–48.2). At the time of final analysis, 19 (54.3%) patients had died due to disease progression. Median OS following the initiation of DEC regimen was 17.8 months (Fig. 1). Fourteen (40.0%) patients had a chemotherapy holiday after 2 consecutive DEC therapy cycles. Median total time of chemotherapy holidays from initial to final DEC therapies was 7.7 months (range 1.7–35.8). The 2-year survival rate following initiation of DEC therapy was 27.1%, while 5-year survival rates following CRPC and initial hormone treatment were 35.2 and 61.0%, respectively.

### Adverse reactions

All ARs were reversible and most were moderate and grade 2 or less. ARs classified as grade 3 or 4 are listed in Table 4. The percentage of grade 3 or 4 ARs for the first cycle was 48.6% (Table 4). Thirteen (38.2%) patients developed grade 3 or 4 leukocytopenia that was managed successfully with granulocyte colony-stimulating factor administration. Red cell or platelet transfusions were given to patients who suffered from grade 4 anemia or thrombocytopenia. One patient died from a non-cancerous cause—aspiration pneumonia.

### Survival analysis

Survival rates were compared between 2 groups divided as shown in Table 5. These parameters were previously described as being poorly prognostic in CRPC [6, 10, 11]. The median OS was significantly shorter in patients with LDH levels of >193 U/L than for those with lower levels (11.5 vs. 29.0 months, $p = 0.001$), and the median OS was significantly shorter for patients with platelet counts of $>25.5\times10^4/\mu L$ than for those with lower counts (13.8 vs. 19.4 months, $p = 0.004$). No significant association was found between other clinical parameters and outcomes. Multivariate analysis including 2 factors (platelet count and LDH level) demonstrated that the LDH level was an independent indicator of survival (odds ratio 6.084, 95% CI 1.650–22.438; $p = 0.007$) (Table 6; Fig. 2).

**Table 2** Patient characteristics

| | *n* (%) |
|---|---|
| Patient age (years) | |
| Median | 68 |
| Range | 54–79 |
| ECOG performance status | |
| 0 | 28 (80.0) |
| 1 | 6 (17.1) |
| 2 | 1 (2.9) |
| Prior therapy | |
| Surgery | |
| Radical prostatectomy | 9 (25.7) |
| Total cystoprostatectomy | 3 (8.6) |
| Radiation | 4 (11.4) |
| Hormone therapy | |
| Combined androgen blockade | 33 (94.3) |
| Diethylstilbestrol | 11 (31.4) |
| Dexamethazone | 15 (42.9) |
| Chemotherapy | |
| Estramustine phosphate | 30 (85.7) |
| Etoposide | 2 (5.7) |
| Differentiation | |
| Well | 0 (0.0) |
| Moderate | 4 (11.4) |
| Poor | 28 (80.0) |
| Unknown | 3 (8.6) |
| Metastatic site | |
| Bone | 30 (85.7) |
| Lymph nodes | 12 (34.3) |
| Visceral | 6 (17.1) |
| PSA at diagnosis (ng/mL) | |
| Median | 95.3 |
| Range | 3–8010 |
| Baseline PSA berore DEC | |
| Median | 99.6 |
| Range | 0.036–4900 |
| Laboratory data at baseline | |
| Hemoglobin (g/dL) | |
| Median | 10.9 |
| Range | 7.2–13.7 |
| Alkaline phosphatase (U/L) | |
| Median | 483.5 |
| Range | 67–4455 |
| Lactate dehydrogenase (U/L) | |
| Median | 193 |
| Range | 103–2052 |
| Platelet counts ($\times 10^4/\mu L$) | |
| Median | 25.5 |
| Range | 9.4–79.4 |
| Cancer pain | |
| Negative | 24 (68.6) |
| Positive | 11 (31.4) |
| Time from CRPC to DEC | |
| Median | 14.9 |
| Range | 1.0–109.1 |

*ECOG* Eastern Cooperative Oncology Group, *PSA* prostate-specific antigen, *DEC* docetaxel, estramustine phosphate and carboplatine therapy, *CRPC* castration-resistant prostate cancer

**Table 3** Clinical outcomes

| | Effective no. of patients/total no. of patients (%) |
|---|---|
| PSA decrease | |
| 30% or greater | 23/35 (65.7) |
| 50% or greater | 16/35 (45.7) |
| 75% or greater | 10/35 (28.6) |
| Measurable disease | |
| PR | 8/12 (66.7) |
| SD | 3/12 (25.0) |

*PSA* prostate-specific antigen, *PR* partial response, *SD* stable disease

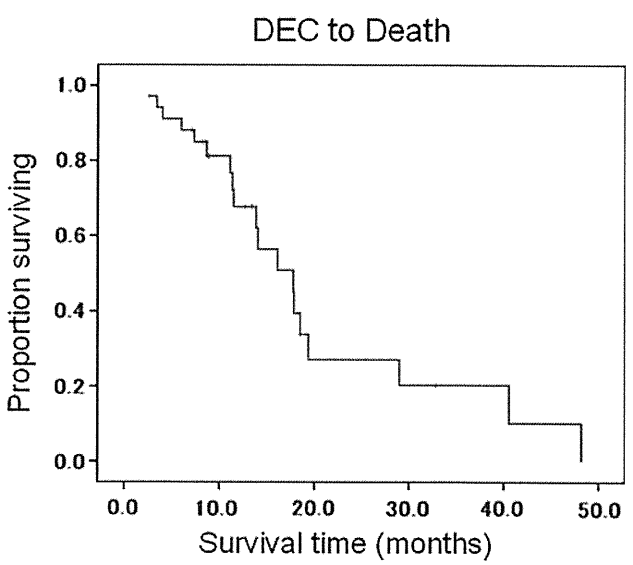


**Fig. 1** Kaplan–Meier estimates of overall survival from initiation of DEC therapy

## Genetic variation affecting adverse reaction

Next, we explored the association between each genotype and severe leukocytopenia. Two (6%) patients were excluded from the SNP study due to non-availability of

**Table 4** Percentage of grade 3 and higher toxic effect at first cycle

| | Grade 3 | % | Grade 4 | % | Total | % |
|---|---|---|---|---|---|---|
| Hematological | | | | | | |
| Anemia | 3 | 8.8 | 0 | 0.0 | 3 | 8.8 |
| Leukopenia | 10 | 29.4 | 3 | 8.8 | 13 | 38.2 |
| Thrombocytopenia | 1 | 2.9 | 1 | 2.9 | 2 | 5.9 |
| Febrile neutropenia | 1 | 2.9 | 0 | 0.0 | 1 | 2.9 |
| Stomatitis | 1 | 2.9 | 0 | 0.0 | 1 | 2.9 |
| Anorexia | 1 | 2.9 | 0 | 0.0 | 1 | 2.9 |
| Pneumonitis | 0 | 0.0 | 1 | 2.9 | 1 | 2.9 |
| Diarrhea | 1 | 2.9 | 0 | 0.0 | 1 | 2.9 |
| Transamirase | 2 | 5.9 | 0 | 0.0 | 2 | 5.9 |

**Table 5** Univariate analysis of prognostic factors in patients with castration-resistant prostate cancer treated with DEC

| Factor | Classification | *p* |
|---|---|---|
| Age (years) | >68 versus ≤68 | 0.647 |
| Initial PSA (ng/mL) | >91 versus ≤91 | 0.212 |
| Baseline PSA (ng/mL) | >115 versus ≤115 | 0.423 |
| CRPC to DEC (months) | >15 versus ≤15 | 0.284 |
| Pretreatment | | |
| Dexamethazone | Positive versus negative | 0.138 |
| Estramustine | Positive versus negative | 0.165 |
| Initial stage | c versus d | 0.569 |
| PSA response (ng/mL) | Positive versus negative | 0.158 |
| | >30 versus ≤30 | 0.181 |
| | >50 versus ≤50 | 0.447 |
| | >75 versus ≤75 | 0.594 |
| Laboratory data | | |
| Hemoglobin (g/dL) | >11 versus ≤11 | 0.18 |
| Alkaline phosphatase (U/L) | >484 versus ≤484 | 0.436 |
| Lactate dehydrogenase (U/L) | >193 versus ≤193 | 0.001 |
| Platelet counts ($\times 10^4/\mu L$) | >25.5 versus ≤25.5 | 0.004 |
| Metastasis | | |
| Skeletal only | Positive versus negative | 0.461 |
| Extra-osseous only | Positive versus negative | 0.652 |
| Skeletal plus extraosseous | Positive versus negative | 0.744 |
| ECOG-PS | 0 versus >0 | 0.741 |
| Time from initiation of hormonal therapy to CRPC (months) | >17.7 versus ≤17.7 | 0.489 |
| Time from initiation of hormonal therapy to administration of DEC (months) | >36 versus ≤36 | 0.095 |
| Pain | Positive versus negative | 0.926 |

*PSA* prostate-specific antigen, *CRPC* castration-resistant prostate cancer, *DEC* docetaxel, estramustine phosphate and carboplatine therapy, *ECOG* Eastern Cooperative Oncology Group

DNA samples. Allelic distribution is listed in Table 7. On univariate analysis, patients carrying the TT genotype of the *ABCB1* C3435T polymorphism had significantly more severe leukocytopenia during the first cycle of DEC therapy ($p = 0.037$). In the multivariate model including all clinical and genetic variables used in the univariate analysis, the TT genotype of the *ABCB1* C3435T polymorphism was an independent predictor of severe leukocytopenia for the first cycle (odds ratio 14.537, 95% CI 1.253–824.316; $p = 0.036$) (Table 8). There was no significant association between any genotype of the 8 SNPs and OS.

## Discussion

Regan et al. [4] reported on a pooled analysis that included 7 trials of TEC therapy. The pooled analysis showed that

**Table 6** Multivariable model predicting overall survival duration

| | Hazard ratio | 95% CI | *p* |
|---|---|---|---|
| Lactate dehydrogenase (U/L) | | | |
| >193 | 6.084 | 1.650–22.438 | 0.007 |
| ≤193 | | | |
| Platelet counts ($\times 10^4/\mu L$) | | | |
| >25.5 | 3.143 | 0.941–10.501 | 0.630 |
| ≤25.5 | | | |

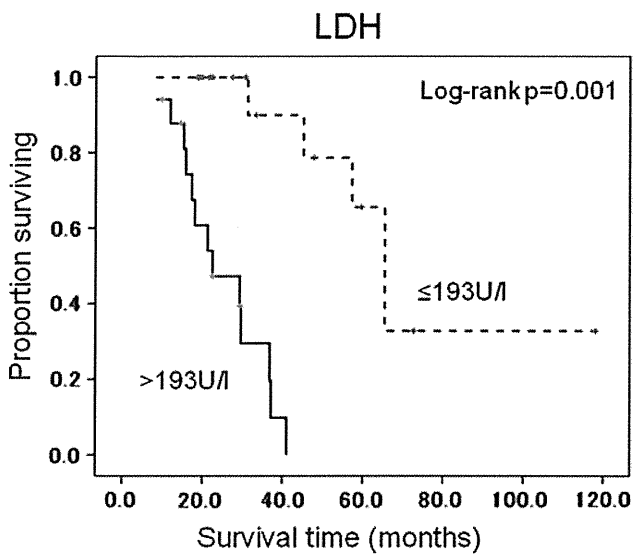


**Fig. 2** Kaplan–Meier survival curves according to serum LDH level

the proportion of patients achieving a PSA response of >50% decrease from baseline was 69%, and that the estimated median survival was 18 months [4]. Kikuno et al. reported the results of a combination chemotherapy that consisted of a 4-week cycle of docetaxel (30 mg/m$^2$ weekly), EMP (10 mg/kg daily) and carboplatin (AUC 6). The outcome of their study was extremely positive, with 95% PSA response and median survival of 26.6 months [12], which appears to be much better than the results of our study. However, the length of the pre-treatment period and amount of medication given to patients in the study conducted by Kikuno et al. seem to have been shorter and lesser, respectively, than those in our study. For example, EMP was administered prior to DEC regimen in 15.0% patients in their study, but in 85.7% in our study. Therefore, it is difficult to assess the superiority of either regimen because of differences in patient characteristics and the timing of initiation of DEC regimen. Our results suggest that DEC may have a potential effect on patients with CRPC, even at the later more advanced stage, and on those who had been pre-treated with EMP.

Based on the concept of conservative treatment with minimum deterioration of QOL, intermittent chemotherapy

**Table 7** Allelic distributions of 8 SNPs

| | Grade 3 or 4 leukocytopenia, *n* (%) | |
|---|---|---|
| | − | + |
| Total | 17 | 16 |
| *MAP4* | | |
| CC | 11 (64.7) | 11 (68.8) |
| CT + TT | 5 (29.4) | 4 (25.0) |
| *MAPT* | | |
| CC | 8 (47.1) | 5 (31.3) |
| CA + AA | 9 (52.9) | 11 (68.8) |
| *ABCG2* | | |
| AG + GG | 15 (88.2) | 14 (87.5) |
| AA | 1 (5.9) | 1 (6.3) |
| *CYP3A5* | | |
| *3/*3 | 10 (58.8) | 11 (68.8) |
| *1/*3 + *1/*1 | 6 (35.3) | 4 (25.0) |
| *XRCC1*-A194G | | |
| CC + CT | 13 (76.5) | 13 (81.3) |
| TT | 4 (23.5) | 3 (18.8) |
| *XRCC1*-C399T | | |
| GG | 11 (64.7) | 8 (50.0) |
| AG + AA | 6 (35.3) | 8 (50.0) |
| *ABCB1*-intron | | |
| GG | 6 (35.3) | 8 (50.0) |
| CG + CC | 11 (64.7) | 8 (50.0) |
| *ABCB1*-C3435T | | |
| CC+CT | 16 (94.1) | 10 (62.5) |
| TT | 1 (5.9) | 6 (37.5) |

is a promising option for patients with CRPC receiving chemotherapy. In a large multi-institutional trial to assess the efficacy of a combination chemotherapy with docetaxel and high-dose calcitriol, Beer et al. [13] reported that most patients took chemotherapeutic holidays and 45.5% patients showed a >50% PSA decline at the time of re-treatment. Because the DEC regimen appears to be associated with higher ARs, along with a higher response rate than the docetaxel regimen with or without EMP, the DEC regimen may not suitable as a continuous therapy. In this study, 40% patients took a chemotherapy holiday with a mean duration of 7.7 months. Further analysis is warranted to compare the QOL of patients having undergone intermittent versus continuous combination chemotherapy with DEC.

It is important to identify which categories of patient are most likely to benefit from docetaxel-based chemotherapy. In the study by Oh et al. [4], extra-skeletal metastases, ECOG-PS, hemoglobin, serum LDH and ALP levels were associated with poor patient survival rates on multivariate analysis. In addition to the LDH level, the univariate analysis indicates that platelet count is a potential marker in detecting poor survival rates in patients with CRPC

**Table 8** Univariate and multivariate analysis of predictive factors for grade 3 or higher leukocytopenia throughout DEC

| Factor | | Category | Odds ratio | 95% CI | *p* |
|---|---|---|---|---|---|
| Univariate analysis | | | | | |
| Age | | Older than 68 versus younger than 68 | 1.111 | 0.262–4.719 | 0.886 |
| *MAP4* | rs56313601 | CT + TT versus CC | 1.250 | 0.263–5.936 | 0.779 |
| *MAPT* | rs3744460 | CC versus CA + AA | 2.139 | 0.472–9.699 | 0.458 |
| *ABCG2* | rs2231137 | AA versus AG + GG | 1.071 | 0.061–18.82 | 0.962 |
| *CYP3A5* | rs776746 | *1/*3 + *1/*1 versus *3/*3 | 1.077 | 0.132–8.797 | 0.945 |
| *XRCC1*-C194T | rs1799782 | TT versus CC + CT | 2.167 | 0.334–14.057 | 0.654 |
| *XRCC1*-A399G | rs25487 | GG versus GA + AA | 2.514 | 0.581–10.882 | 0.285 |
| *ABCB1*-C3435T | rs1045642 | TT versus CC + CT | 10.000 | 1.030–97.044 | 0.037 |
| *ABCB1*-intron | rs7779562 | GG versus GC + CC | 1.905 | 0.454–7.983 | 0.479 |
| Multivariate analysis | | | | | |
| Age | | Older than 68 versus younger than 68 | 1.783 | 0.203–15.625 | 0.602 |
| *MAP4* | rs56313601 | CT + TT versus CC | 1.082 | 0.133–8.783 | 0.941 |
| *MAPT* | rs3744460 | CC versus CA + AA | 1.007 | 0.144–7.058 | 0.994 |
| *ABCG2* | rs2231137 | AA versus AG + GG | 2.144 | 0.036–127.539 | 0.714 |
| *CYP3A5* | rs776746 | *1/*3 + *1/*1 versus *3/*3 | 1.083 | 0.067–17.458 | 0.955 |
| *XRCC1*-C194T | rs1799782 | TT versus CC + CT | 4.247 | 0.172–105.138 | 0.377 |
| *XRCC1*-A399G | rs25487 | GG versus GA + AA | 2.863 | 0.418–19.622 | 0.284 |
| *ABCB1*-C3435T | rs1045642 | TT versus CC + CT | 32.141 | 1.253–824.316 | 0.036 |
| *ABCB1*-intron | rs7779562 | GG versus GC + CC | 1.670 | 0.192–14.537 | 0.642 |

treated with DEC therapy. Thrombocytosis is known to be present in a wide range of malignancies, with a reported incidence of 10–57% [14]. Interestingly, Helley et al. [15] suggested that the level of platelet microparticles, which may affect tumour chemotaxins, adhesion and proliferation, was a potential prognostic factor in patients with CRPC undergoing docetaxel-based chemotherapy.

The individual variation in therapeutic effect may be due to the biological characteristics of the cancer itself and the innate properties of the patient as the cancer host. These variations are partially explained by genetic polymorphism, as represented by SNPs. Although the sample size was small, we found that the TT genotype of the *ABCB1* C3435T polymorphism was an independent predictor of severe leukocytopenia in patients with CRPC treated with DEC regimen. ABCB1 is responsible for a large portion of the systemic efflux capacity towards docetaxel. ABCB1 expression and protein folding are reported to be largely influenced by 3 SNPs including C3435T [16]. Sissung et al. [17] revealed that these variations correlated with survival, neutropenia and peripheral neuropathy in prostate cancer patients. Although further study is warranted to assess other *ABCB1* SNPs that are known to be associated with drug metabolism, our results support the view that the genotype of *ABCB1* SNPs has significant association with ARs induced by docetaxel-based chemotherapy.

In spite of limited sample size, the results of this phase II study evaluating DEC regimen showed that it has a high response rate and potential survival benefits in late-stage CRPC. We have demonstrated a significant impact of LDH levels on prediction of overall survival. Furthermore, the *ABCB1* C3455T polymorphism was associated with severe leukocytopenia, indicating that genotype analysis of the *ABCB1* polymorphism may be useful in predicting severe leukocytopenia in patients undergoing combination chemotherapy with DEC.

**Acknowledgments** We thank Yukiko Sugiyama and Yoko Mitobe for their excellent technical assistance.

**Conflict of interest** No author has any conflict of interest.

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JOURNAL OF LAPAROENDOSCOPIC & ADVANCED SURGICAL TECHNIQUES
Volume 21, Number 6, 2011

DOI: 10.1089/lap.2010.0457

# What Is the Most Preferred Wound Site for Laparoscopic Donor Nephrectomy?: A Questionnaire Assessment

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## Abstract

***Introduction:*** Although specimen extraction site selection for laparoscopic donor nephrectomy (LDN) is relatively flexible and is mostly selected by surgeons from the patient's standpoint, the patient's request may differ from the medical worker's recommendation. The cosmetic aspect may also differ with age, gender, and the extent of medical knowledge. We performed an unsigned questionnaire assessment of individual preferences for LDN wound sites.

***Materials and Methods:*** Between August 2007 and October 2008, we surveyed LDN wound site preferences among 148 physicians, 263 nurses, and 266 outpatients of urology at Akita University Hospital. They were questioned for their age, gender, occupation (medical worker or not), and for the most preferred surgical wound site among the following: A, lower vertical midline: B, upper vertical midline: C, anterior subcostal: D, Pfannenstiel: E, Gibson: and F, subcostal flank. The valid response rate was 93.5% (677/724).

***Results:*** Wound sites preferred (ranked in descending order) were F (48.3%), D (25.6%), E (10.5%), A (9.0%), C (5.2%), and B (1.4%). The subcostal flank incision was the most preferred in almost all the categories. Second preferences were Pfannenstiel incisions in women and incisions on the lower abdomen in men. Overall, flank and lower abdominal incisions tended to be preferred, and mid and upper abdominal incisions tended to be avoided. Medical workers selected the subcostal flank and Pfannenstiel incisions more frequently than outpatients. With increasing age, the selection rates of the Gibson and the lower vertical midline incisions increased, whereas the subcostal flank and the Pfannenstiel incisions decreased.

***Conclusions:*** The subcostal flank was the most preferred LDN sites. Age, gender, and the extent of medical knowledge may influence the individual preferences for LDN wound sites.

## Introduction

The selection of specimen extraction site for laparoscopic donor nephrectomy (LDN) is relatively flexible. Recently, a modified Pfannenstiel incision has been popular as a specimen extraction site for pure LDN.[1] In addition, the Pfannenstiel incision is reported to have an outstanding cosmetic advantage.[2,3] However, whether living kidney donors will actually choose the Pfannenstiel incision as the specimen extraction site for LDN remains undetermined. Although surgeons mostly select the specimen extraction site from the patient's standpoint, the patients' request may differ from the medical service worker's recommendation. In addition, the cosmetic aspect of surgical wound site selection may differ with age, gender, and the extent of medical knowledge. Here, we performed an unsigned questionnaire assessment of individual preferences for LDN wound sites. This is the first report investigating individual preferences for LDN wound sites.

## Materials and Methods

Between August 2007 and October 2008, we surveyed of relative LDN wound site preferences among 148 physicians and 263 nurses working at Akita University Hospital, and 266 outpatients who visited urology clinics in the hospital. The subjects were aged between 18 and 75 years (mean 43.0 ± 16.5). They were questioned for their age, gender, occupation (medical worker or not), and for the most preferred

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surgical wound site among the following: A, lower vertical midline incision: B, upper vertical midline incision: C, anterior subcostal incision: D, Pfannenstiel incision: E, Gibson incision: and F, subcostal flank incision (Fig. 1). The questionnaire in this study did not include the contents regarding the past history of abdominal surgery. We analyzed the questionnaire results according to gender, occupation, and generation (young, 18–35 years: middle, 36–55 years: and senior, 56–75 years). The valid response rate to the questionnaire was 93.5% (677/724). The mean ages of all subjects, medical workers (physicians and nurses), and outpatients were 44.8±18.3, 35.4±10.9, and 54.8±16.8 years, respectively. The ratios of man to woman in all subjects, medical workers, and outpatients were 276:401, 120:291, and 156:110, respectively. The protocol for the present study was approved by the Research Committee at our institute.

## Results

The most preferred wound sites were ranked in descending order as follows: F, subcostal flank incision (48.3%): D, Pfannenstiel incision (25.6%): E, Gibson incision (10.5%): A, lower vertical midline incision (9.0%): C, anterior subcostal incision (5.2%): and B, upper vertical midline incision (1.4%) (Fig. 2a-1). Overall, surgical wound sites on the flank and lower abdomen tended to be preferred, and those on the mid and upper abdomen tended to be avoided. The subcostal flank incision was the most preferred site in almost all categories (men, women, medical workers, and outpatients) (Fig. 2). The second preference sites were Pfannenstiel incisions in women and incisions on the lower abdomen in men (Fig. 2a-2, 3). Most medical workers selected the subcostal flank and the Pfannenstiel incisions, and most men preferentially selected the subcostal flank incision, whereas an equal number of women selected the subcostal flank and the Pfannenstiel incisions (Fig. 2b). Female outpatients preferred the Pfannenstiel incision next to the subcostal flank incision, whereas male outpatients selected various surgical wound sites (Fig. 2c-2, 3).

Comparing generations, the subcostal flank and the Pfannenstiel incisions were preferentially selected by the young generation (Fig. 3a-1). The selection rates of Gibson and the lower vertical midline incisions increased with age, whereas those of the subcostal flank and the Pfannenstiel incisions decreased (Fig 3a-1). In young and middle generations of overall and medical workers, a large number of women selected the Pfannenstiel incision as the most preferred site, whereas most men selected the subcostal flank incision (Fig. 3a-2, 3, 3b-2, 3). The selection rates of lower vertical midline and Gibson incisions in male and female outpatients, respectively, increased with age.

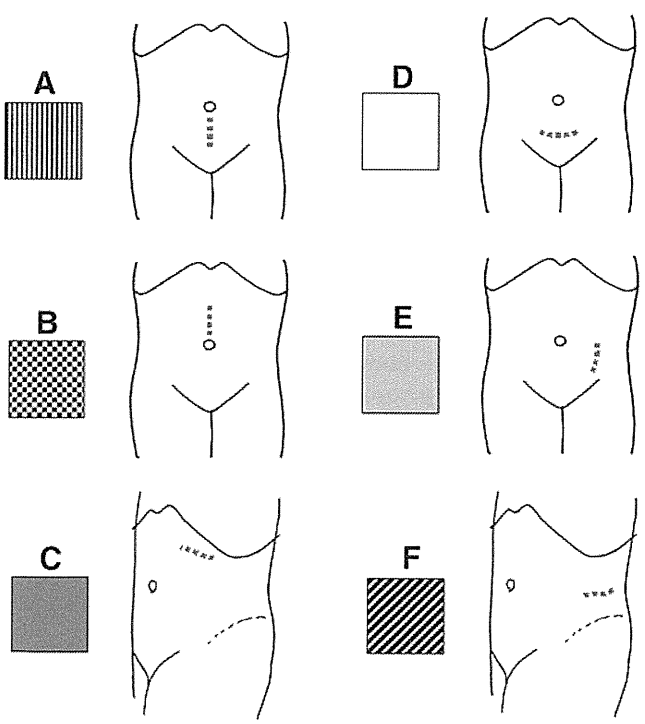


FIG. 1. Scheme of the specimen extraction sites for living donor nephrectomy. The questionnaire including questions pertaining to age, gender, occupation (medical worker or not), and the most preferable surgical wound site among the followings: **A**, lower vertical midline incision; **B**, upper vertical midline incision; **C**, anterior subcostal incision; **D**, Pfannenstiel incision; **E**, Gibson incision; and **F**, subcostal flank incision.

## Discussion

Recently, LDN has been widely accepted for many kidney transplant protocols because of its minimal invasiveness and cosmetic perspective compared with open donor nephrectomy.[4–7] The quality of life of the LDN donor is better than that of the open donor nephrectomy donor.[7,8] In addition, in donor nephrectomy, an anterior vertical mini-incision is more preferred due to probable cosmetic satisfaction, reduced pain, and complications compared with an open flank incision.[9,10] For these reasons, surgeons with sufficient laparoscopic or mini-incisional (endoscopic minilaparotomy) surgical skill should adapt their operative procedure to living donors. On the other hand, the specimen extraction site for LDN is relatively flexible. However, no discussion has been made regarding the most preferred LDN wound site for living donors.

In general, we recognize that patients, especially young women, prefer the Pfannenstiel incision also called the "bikini cut" when they undergo an abdominal operation. In this study, however, the subcostal flank incision was most preferentially selected in almost all categories, including young women. Overall, surgical wound sites on the flank and lower abdomen tended to be preferred, and those on the mid and upper abdomen tended to be avoided. We demonstrate a case of hand-assisted LDN at our institute in which the surgical wound scar on the lateral abdomen was almost invisible from the front (Fig. 4). The front view of the surgical wound scar may have strongly impacted the cosmetic aspect in this study. It remains unknown whether the preference for the subcostal flank incision in Japanese population holds true for Western or other ethnic populations.

The selection rate of the Pfannenstiel incision was about one-quarter, that is, second in the rank. Women selected the Pfannenstiel incision more often than men, and this incision was most supported by women in the middle generation. Most of the young generation selected the subcostal flank and the Pfannenstiel incisions, and the selection rates of surgical wound sites on the lower abdomen increased with age. Although medical workers selected the Pfannenstiel incision as

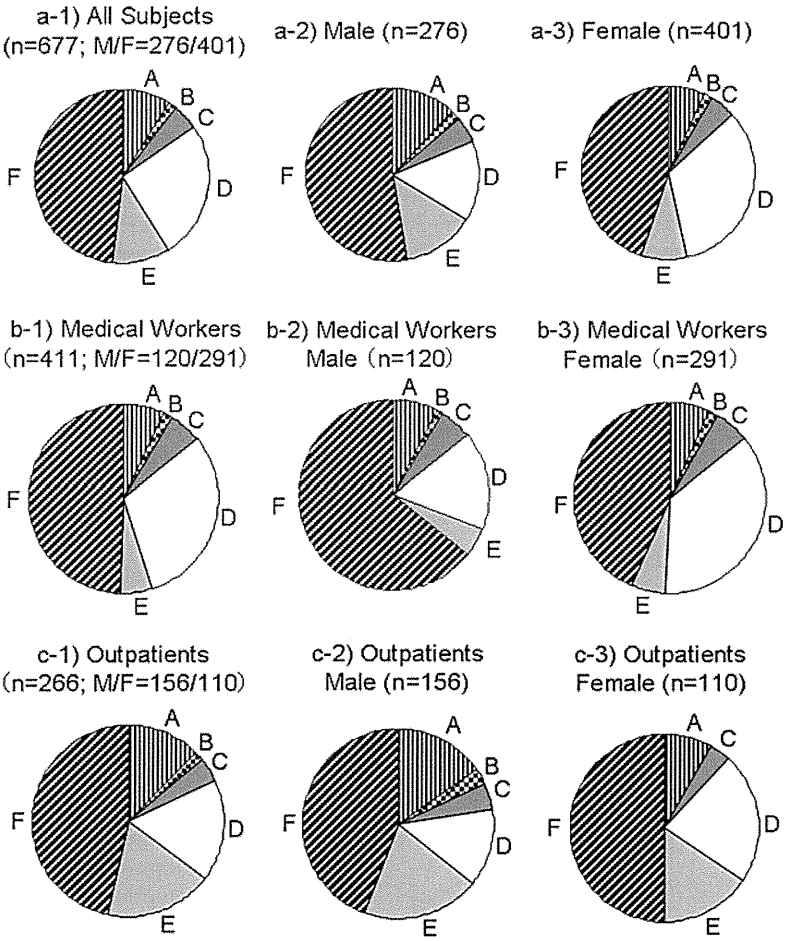


**FIG. 2.** Preferable wound site ranking for living donor nephrectomy. **a-1**, All subjects; **a-2**, men; **a-3**, women; **b-1**, medical workers; **b-2**, medical workers (men); **b-3**, medical workers (women); **c-1**, outpatients; **c-2**, outpatients (men); **c-3**, outpatients (women).

the second preference, outpatients preferred surgical wound sites on the lower abdomen. The reasons why female medical workers selected the Pfannenstiel incision more preferentially than outpatient women are not clear. However, medical workers might know that the Pfannenstiel incision is generally associated with superior cosmesis and less pain. Generally, subjects in the general population do not have such knowledge regarding the superiority of the Pfannenstiel incision. In addition, senior people have a tendency to select a familiar surgical wound site, such as the Gibson and/or the lower vertical midline incisions. The difference observed in terms of wound site selection between male medical workers and outpatient men was influenced strongly by the age gap. Age, gender, and extent of medical knowledge influenced the selection of LDN wound sites.

Whether complication rates differ among surgical wound sites is controversial. Tisdale et al. compared the Pfannenstiel incision to expanded port site incisions (extending the inferior working 10-mm port sites lateral to the rectus abdominis muscle, transversely) as intact specimen extraction site during laparoscopic nephrectomy procedures.[11] They reported that the Pfannenstiel incision was less morbidity, because only one patient in the Pfannenstiel incision group developed cellulitis at the extraction site, whereas three patients in the expanded port site group developed incisional hernia.[11] However, it should be taken into consideration that all body mass indexes of these three patients were over 35. Bird et al. reported that in patients with a high body mass index using a paramedian extraction site at laparoscopic radical nephrectomy is a significant risk factor for incisional hernia formation.[12] Obesity may be a stronger factor of incisional hernia than the wound sites. Meanwhile, Gupta et al. reported that the iliac fossa incision (extending the spinoumblical port incision, close to Gibson incision) had less morbidity than the Pfannenstiel incision.[13] Gill et al. reported no difference in morbidity or recovery between the Gibson and the flank incisions for intact specimen extraction during laparoscopic nephrectomy procedures.[14] In a randomized study, surgical wound complication rates among midline, para-midline vertical, and transverse incisions were not different.[15] In addition, no randomized trial has been performed to investigate the difference in wound pain among surgical wound sites, and, therefore, further investigation will be needed. Although the subcostal flank incision may be associated with strong wound pain if the three muscle layers are divided, the muscle splitting technique will reduce wound pain and the wound hernia rate.[16]

This study has the limitations that the results for outpatients who visited urology clinics in our hospital were

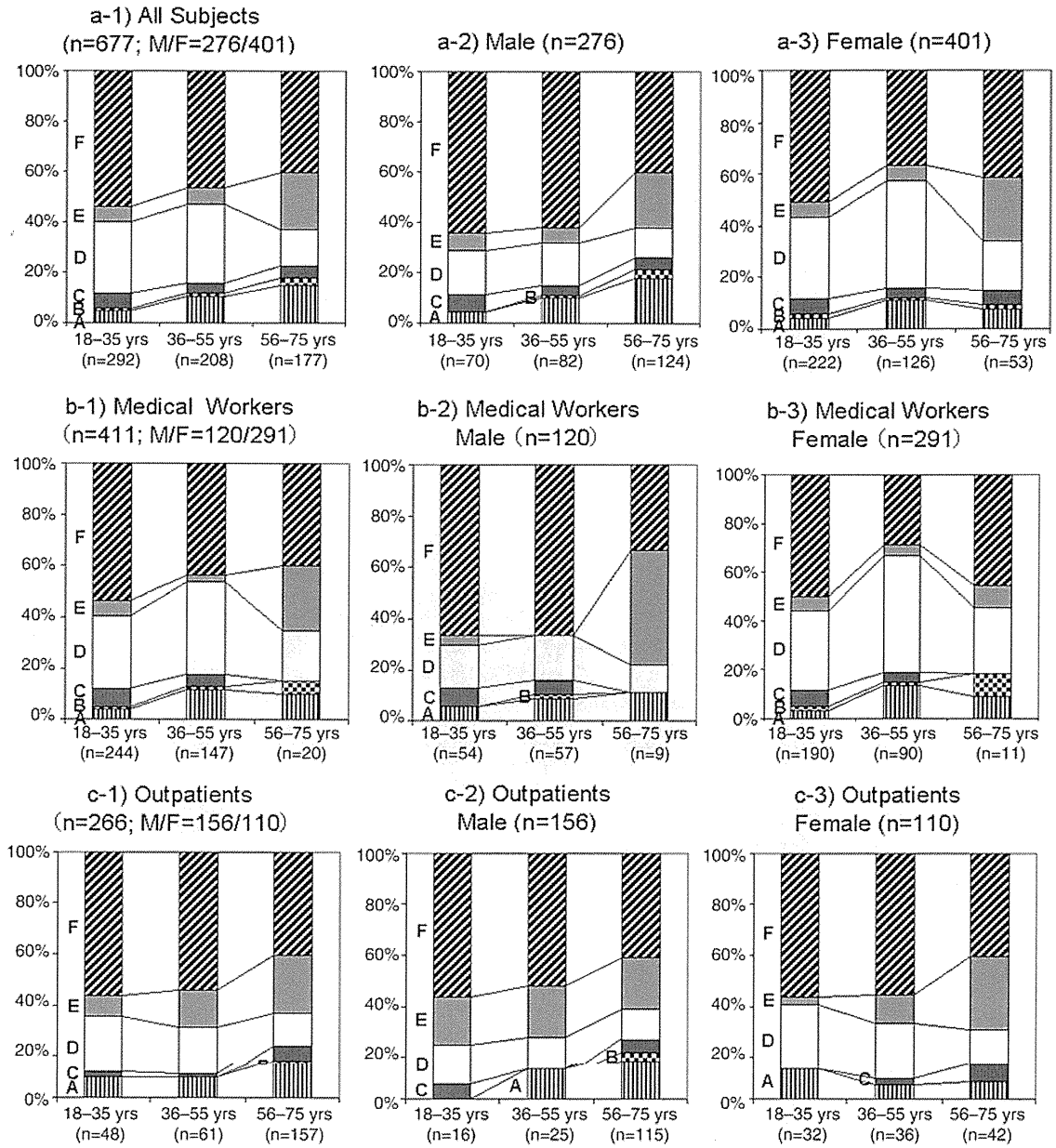


**FIG. 3.** Preferable wound site ranking in different generations. Subjects were divided into three generations (young, 18–35 years: middle, 36–55 years: senior, 56–75 years). The type of the wound site is as described in Figure 1.

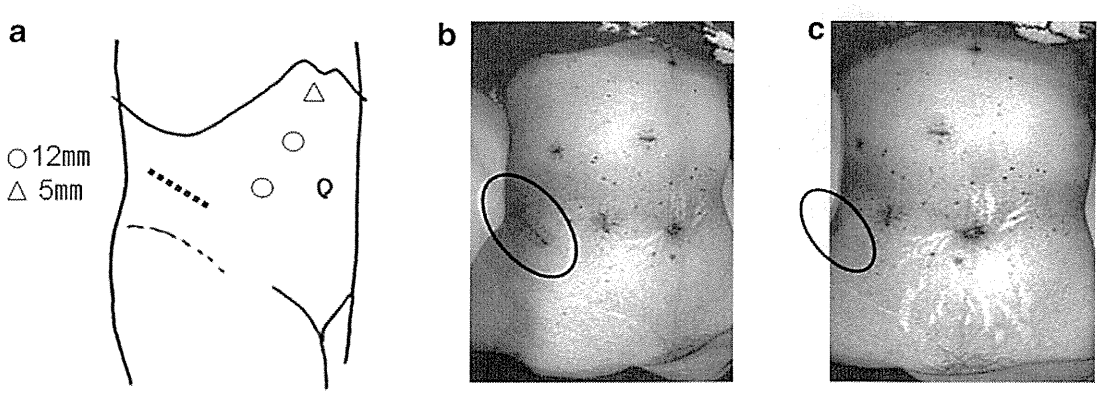


**FIG. 4.** Scheme of port and extraction sites in laparoscopic right hand-assisted donor nephrectomy at our institute **(a)**. One case of wound scar after laparoscopic right hand-assisted donor nephrectomy **(b**, oblique view: **c**, front view). The wound scar after the flank incision is not noticeable in front view **(b, c)**.

regarded as general outpatient data, and subjects may feel less reality from the schemas of the wound position. In addition, subjects' responses may be affected by information about the degree of postoperative pain and the occurrence rates of nerve injury, muscle atrophy, and abdominal incisional hernia for each surgical wound site. Since this questionnaire provided no information regarding pain and complication rates for the different surgical wound sites, our questionnaire may be regarded as unbiased, and the subjects other than medical workers might have chosen the site of incision from the standpoint of pure cosmetic preference. As noted above, differences in complication rates among surgical wound sites have not been clarified.[11–15] Although the present study indicated that the subcostal flank incision may be superior from the standpoint of cosmesis and patient preference, further randomized clinical study on a large scale is required to establish whether an incision made with a meticulous muscle splitting technique is associated with a lower complication rate and less wound pain than other incisions.

The quality of life of living donors may improve if they can select the surgical wound site themselves. A pure laparoscopic procedure does not limit the specimen extraction site, whereas hand-assisted LDN and/or mini-incision donor nephrectomy (endoscopic minilaparotomy) is comparatively limiting.

## Conclusion

The subcostal flank incision was more preferred over the Pfannenstiel or other incisions as the specimen extraction site for LDN. Age, gender, and extent of medical knowledge influenced the selection of LDN wound sites. Further study is needed to establish whether subcostal incisions are associated with less morbidity, wound pain, good cosmesis, and patient preference.

## Disclosure Statement

No competing financial interests exist.

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Int J Clin Oncol
DOI 10.1007/s10147-010-0154-6

CASE REPORT

# A case study of metastatic Xp11.2 translocation renal cell carcinoma effectively treated with sunitinib

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Received: 1 September 2010 / Accepted: 16 November 2010


**Abstract** We report a case of Xp11.2 translocation renal cell carcinoma (RCC) whose lung metastases were effectively treated with sunitinib. A 43-year-old woman presenting with upper abdominal pain was diagnosed with a left renal tumor. Laparoscopic left radical nephrectomy was performed. Histopathological examination of the surgical specimen revealed a clear-cell carcinoma of the left kidney. Two years later, multiple lung metastases were detected and the patient was treated daily with 50 mg sunitinib. A computed tomography scan performed after 2 cycles of sunitinib treatment revealed partial regression of these metastases. The partial regression has been maintained for >3 years. In retrospective evaluation of the primary RCC, tumor cells showed strong nuclear staining for transcription factor E3 (TFE3) protein and *TFE3* split-fluorescence in-situ hybridization revealed translocation involving the *TFE3* gene. These findings strongly support diagnosis of Xp11.2 translocation RCC.

**Keywords** Renal cell carcinoma · Xp11.2 translocation · Sunitinib · Fluorescence in situ hybridization

## Introduction

Xp11.2 translocation renal cell carcinoma (RCC) is reported to be common in children and believed to be extremely rare in adults. Argani et al. [1] reported 28 adult cases of Xp11.2 translocation RCC with strong female predominance (female to male ratio, 22:6) and a tendency to spread to perirenal lymph nodes. However, little is known about the natural behavior of this tumor, and treatment strategies have not yet been established. This subset of RCC is characterized by various translocations involving chromosome Xp11.2 that result in gene fusion involving the *transcription factor binding E3* (*TFE3*) gene. In a large randomized study, administration of sunitinib, an orally active multikinase inhibitor, resulted in significant prolongation of progression-free survival (PFS) of patients with clear-cell histology of RCC [2]. However, evidence for its efficacy in non-clear-cell histology, especially Xp11.2 translocation RCC, is lacking. We report the case of a patient with metastatic Xp11.2 translocation RCC who achieved an excellent therapeutic response to sunitinib treatment. We further discuss the mechanisms of therapeutic response to sunitinib treatment.

## Case report

A 43-year-old woman who presented with sudden onset of upper abdominal pain is the subject of this study. Ultrasonography and computed tomography (CT) revealed a left renal mass measuring 10 cm in diameter with a tumor thrombus in the left renal vein (Fig. 1). She was diagnosed with RCC of clinical stage T3bN0M0, and laparoscopic radical nephrectomy was performed without complications. Her perioperative period was uneventful. Histopathological examination of the surgical specimen revealed a clear-cell carcinoma (Fig. 2), G2 > G3, INF alpha, and pT3bpN0 with a negative surgical margin. The patient developed multiple lung metastases (maximum diameter 1.0 cm)

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Published online: 15 December 2010

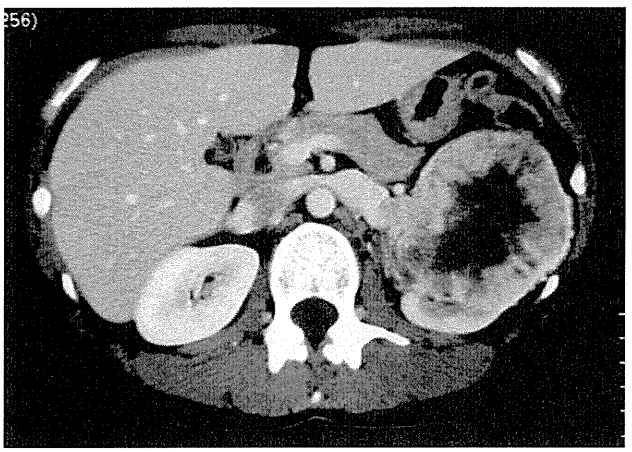

**Fig. 1** Computed tomography reveals a left renal mass measuring 10 cm in diameter with a tumor thrombus localized in the left renal vein

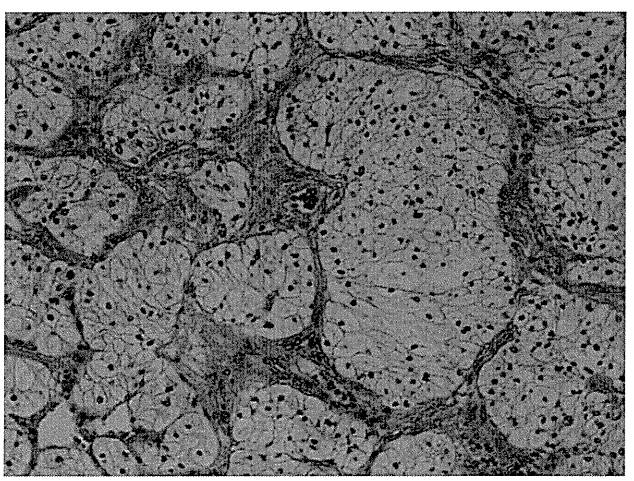

**Fig. 2** H&E staining of the surgical specimen (×200)

2 years after the nephrectomy (Fig. 3a) and was administered 50 mg sunitinib daily. A CT scan performed 6 weeks after the initiation of treatment confirmed the partial regression of the lung metastases (Fig. 3b). Forty-two months after initiation of the sunitinib treatment, the partial regression has been maintained (Fig. 3c). Dose reduction to 37.5 mg/day was required in subsequent cycles because of fatigue, and the patient is being treated with the same sunitinib dosage without major adverse effects.

We performed a retrospective evaluation of the primary RCC tumor because of the differential diagnosis of Xp11.2 translocation RCC that was added to the new World Health Organization classification in 2004. The tumor cells showed strong nuclear staining for TFE3 protein (Fig. 4) and were positive for *TFE3* split-fluorescence in-situ hybridization (FISH: Fig. 5). Consequently, this case was diagnosed as Xp11.2 translocation RCC.

## Discussion

Xp11.2 translocation RCC, which was originally reported to have papillary architecture and clear cytoplasm, is often confused with clear-cell or papillary RCC, probably because appropriate diagnostic methods are not performed to confirm the diagnosis. In recent years, various forms of Xp11.2 translocation RCC have been identified and characterized at the morphological and molecular levels [3]. Such translocations result in gene fusion between the *TFE3* gene located on chromosome Xp11.2 and various partner genes. Argani et al. [1] reported that nuclear immunoreactivity for TFE3 protein is a highly sensitive and specific assay for neoplasm bearing *TFE3* gene fusion. The antibody used for this procedure recognizes the C-terminal portion of the protein, which is retained in all TFE3 fusion proteins. The native TFE3 protein is known to be expressed ubiquitously but is undetectable in normal tissues by immunohistochemical analysis. It is believed that different *TFE3* gene fusion consistently lead to overexpression of the protein [1]. However, some authors have pointed out that the TFE3 antibody is only moderately reliable [4]. A FISH assay performed on formalin-fixed, paraffin-embedded tissue is known to be more sensitive in detecting the Xp11.2 translocation [5]. We established the *TFE3* split-FISH assay by utilizing the FISH probes located on both sides of the *TFE3* gene (GSP Research Institute, Kawasaki, Japan). The 5′-fragment and 3′-fragment of the *TFE3* gene were labeled with Texas Red™ and fluorescein isothiocyanate, respectively. Tumor cells with the translocation have a split signal (red and green signals are observed separately), whereas cells without the translocation have a fused signal (Fig. 5). In this case, although karyotype analysis was not performed, the expression of TFE3 protein was confirmed immunohistochemically and the *TFE3* split-FISH analysis showed the translocation involving the *TFE3* gene. This is the first case of Xp11.2 translocation RCC confirmed by the *TFE3* split-FISH assay and also effectively treated with sunitinib.

The TFE3 fusion proteins are believed to bind to the *MET* promoter region, leading to its activation [6]. Up-regulation of *MET* by TFE3 fusion proteins results in strong *MET* autophosphorylation and activation of downstream signaling in the presence of HGF. Tyrosine kinase inhibitors, for example sunitinib, have changed treatment strategies for management of metastatic RCC. However, the activity of sunitinib in non-clear-cell histology has not been fully evaluated. Malouf et al. [7] reported the efficacy of molecule-targeted therapy for Xp11.2 translocation RCC. Twelve Xp11.2 translocation RCC patients with metastases had received targeted therapy (eleven patients received sunitinib, one received mammalian-targeted rapamycin inhibitor), and comparison with cytokine therapy

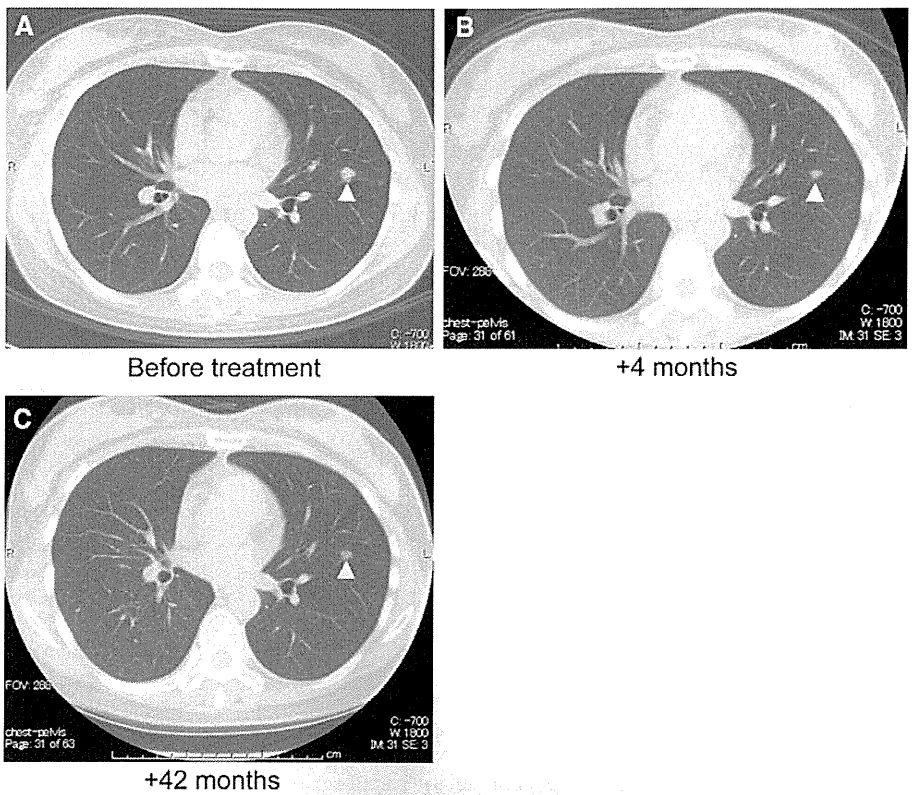


**Fig. 3** Computed tomography reveals lung metastasis (*arrow*) before administration of sunitinib (**a**), 4 months after the initiation of sunitinib (**b**), and 42 months after the initiation of sunitinib (**c**)

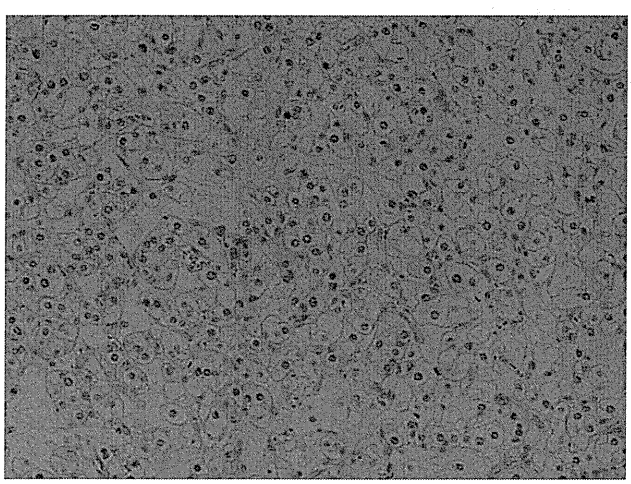

**Fig. 4** Immunohistochemical evaluation revealed tumor cells showing nuclear-specific staining for TFE3 protein

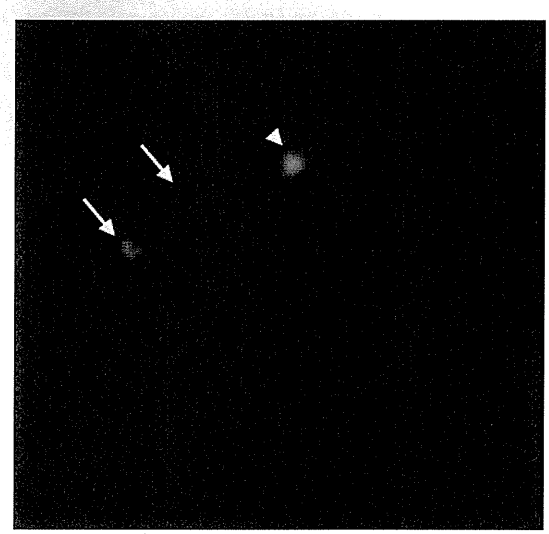

**Fig. 5** Split-fluorescence in-situ hybridization of Xp11.2 translocation renal cell carcinoma. *Arrows* indicate the translocated Xp11.2 fragments, and the *arrowhead* indicates the normal X chromosome

revealed favorable response rate and PFS. However, in their study, diagnosis for most of the patients of Xp11.2 translocation was confirmed by immunohistochemical analysis, and only two were diagnosed cytogenetically. Because immunohistochemical diagnosis of TFE3 protein expression is sometimes difficult and ambiguous, it may be better to define the presence of the Xp11.2 translocation both genetically and immunohistochemically. With such clear distinction, comparison of the clinical characteristics of RCC with Xp11.2 translocation can be achieved.

Possible mechanisms explaining the effect of sunitinib on Xp11.2 translocation RCC tumors include RTK inhibition and direct anti-tumor and anti-angiogenic activity by targeting VEGF, PDGFR, and RET receptors [8–10]. Stacchiotti et al. [11] reported that the anti-tumor activity of sunitinib in alveolar soft part sarcoma, which is also associated with Xp11.2 translocation, may be mediated mainly by PDGFRs. However, results from another study