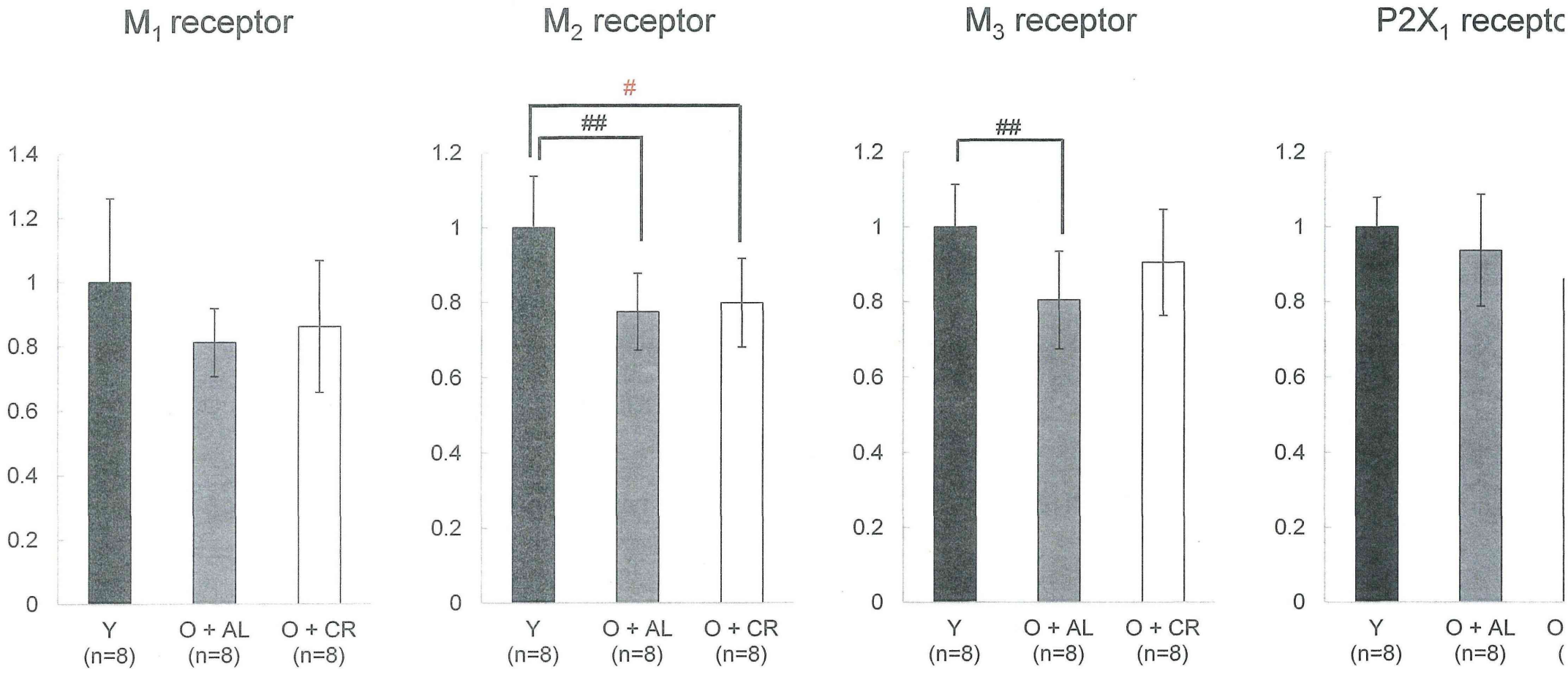
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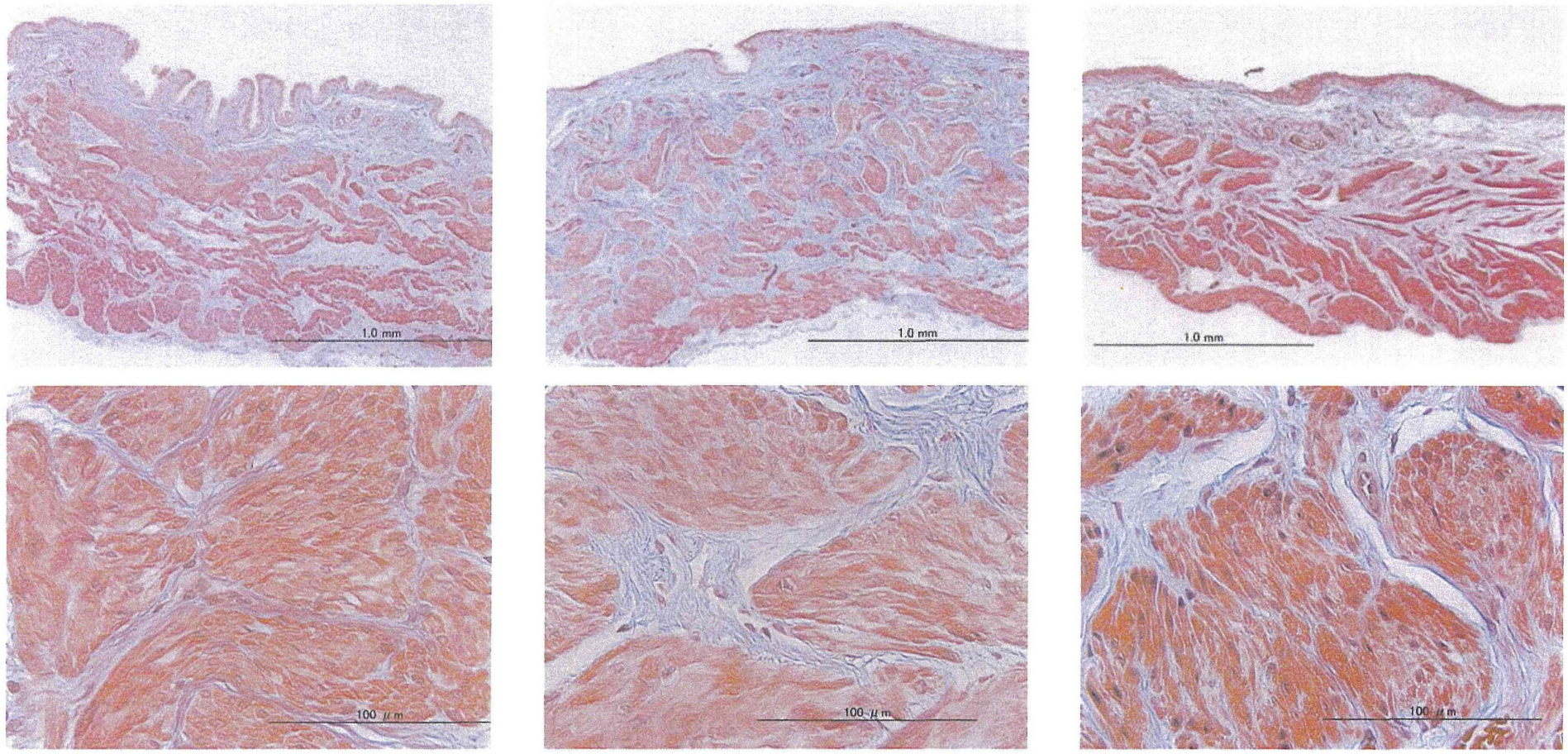
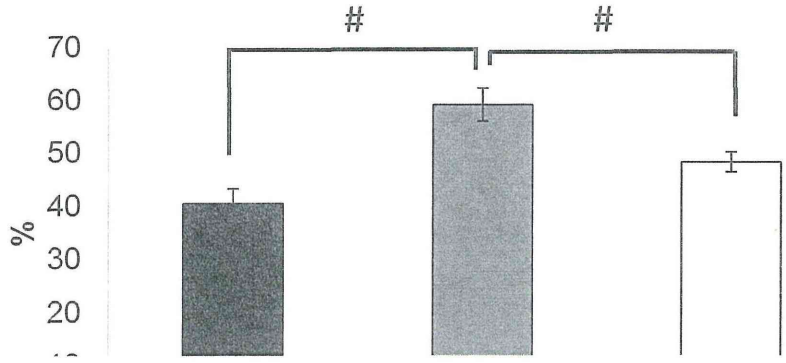
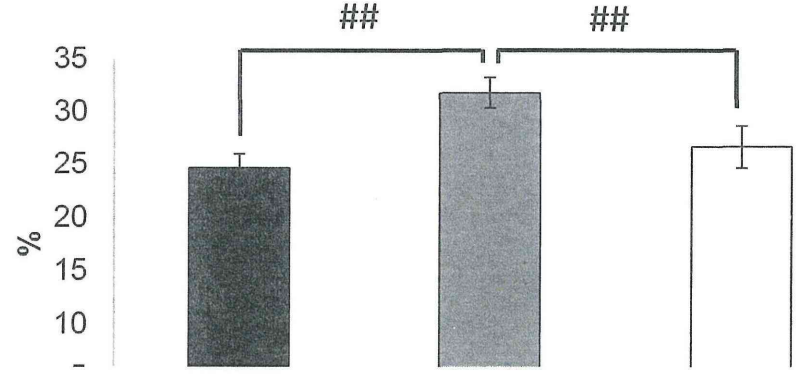


B: detrusor layer



C: whole bladder wall



ABBREVIATIONS

ADH = antidiuretic hormone

ATP = adenosine triphosphate

BUN = blood urea nitrogen

CCh = carbachol

CR = caloric restriction

EFS = electrical field stimulation

E2 = estradiol

FV = frequency volume

HDL- Chol = high density lipoprotein cholesterol

LDL - Chol = low density lipoprotein cholesterol

M_1 = muscarinic 1

M_2 = muscarinic 2

M_3 = muscarinic 3

mATP = α,β-Methylene-ATP

N = number of animals

n = number of detrusor strips

O+AL = old ad libitum fed with normal food

O+CR = old with calorie restriction

RT-PCR= reverse transcription polymerase chain reaction

SEM = standard error of the mean

TTX = tetrodotoxin

Y = young

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Original Article

Original Article

Combination of docetaxel, ifosfamide and cisplatin (DIP) as a potential salvage chemotherapy for metastatic urothelial carcinoma

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Abstract

Objective: The aim of this study was to evaluate the efficacy and toxicity of the combination of docetaxel, ifosfamide and cisplatin as salvage chemotherapy after failure of standard cisplatin-based regimens for metastatic urothelial carcinoma.

Methods: We prospectively administered docetaxel, ifosfamide and cisplatin chemotherapy to patients with metastatic urothelial carcinoma refractory to standard cisplatin-based regimens from 2003 to 2013. Patients who had received only adjuvant and/or neoadjuvant chemotherapy were excluded. Eligible patients received every 28 days docetaxel 60 mg/m^2 on Day 1, ifosfamide 1.0 g/m^2 on Days 2–6 and cisplatin 20 mg/m^2 on Days 2–6. The primary endpoints were progression-free survival and overall survival, calculated from the start of docetaxel, ifosfamide and cisplatin chemotherapy. Secondary endpoints included objective response and related toxicity.

Results: Twenty-six cases received a median of 3.0 cycles of docetaxel, ifosfamide and cisplatin chemotherapy (interquartile range: 2–5), resulting in a median progression-free survival of 3 months (interquartile range: 2–9.5 months) and median overall survival of 8.5 months (interquartile range: 6.5–18.75 months), respectively. Of 26 patients, seven (27%) achieved major treatment responses, with one complete response (4%) and six partial responses (23%). Most of Grade 3/4 toxicities were hematologic events, including leukopenia (77%), anemia (54%) and thrombocytopenia (46%). No death from toxicity was observed.

Conclusions: Our results indicate that docetaxel, ifosfamide and cisplatin chemotherapy is a tolerable and moderately active regimen for metastatic urothelial carcinoma after failure of standard cisplatin-based regimens.

Key words: bladder cancer, urothelial carcinoma, metastatic urothelial carcinoma, salvage chemotherapy

Introduction

Approximately 430 000 patients were newly diagnosed with urothelial carcinoma (UC) of urinary bladder in 2012, resulting in newly 160 000 deaths worldwide (1) and >7000 deaths in Japan (2). Metastatic UC is often intractable with the median survival of ~15 months (3,4). For advanced or metastatic UC, combination of gemcitabine plus cisplatin (GC) or combination of methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) are the standard treatment regimens in the first-line setting (3–6). However, the optimal second-line salvage chemotherapy has not yet been established (7,8). Recent studies have shown that docetaxel monotherapy (9,10) or combination therapies including docetaxel (11–14) can attain moderate anti-cancer activity with manageable toxicity in platinum-resistant UC. Previously, we reported preliminary results on the efficacy and toxicity of combination chemotherapy of docetaxel, ifosfamide and cisplatin (DIP) in 14 patients with metastatic UC (15). DIP chemotherapy yielded 10 partial response cases (72%) with a median duration of response of 6.5 months. Of five MVAC refractory cases, four (80%) showed partial response with a median duration of response of 5.5 months. We therefore undertook this prospective study to evaluate the efficacy and safety of DIP chemotherapy as a potential second-line therapy for metastatic UC.

Patients and methods

The study was conducted according to the Declaration of Helsinki and approved by the ethical committee of our institute (P2003028-11). Written informed consent was obtained from all patients. We prospectively enrolled patients who had been treated with at least one cisplatin-based chemotherapy regimen for metastatic UC from January 2003 to August 2013. Patients were required to have ECOG performance status 0–2, adequate bone marrow function (leukocyte count >3000/μl, hemoglobin > 10.0 g/dl and platelet count >75 000/μl) and adequate renal function (estimated creatinine clearance >30 ml/min, calculated by Cockcroft–Gault's equation (16)). Patients who had received only neoadjuvant and/or adjuvant chemotherapy or who had no measurable metastatic lesions were excluded. Prior local or intravesical therapy, immunotherapy or radiation therapy was allowed if completed at least 4 weeks before enrollment. Other exclusion criteria included active infection, clinically significant cardiac arrhythmia or congestive heart failure, brain metastases, second primary malignancy or clinically intractable pleural effusions or ascites.

For treatment schedule, docetaxel, ifosfamide and cisplatin were given as described previously (15). Briefly, patients received docetaxel 60 mg/m^2 on Day 1, ifosfamide 1.0 g/m^2 (with Mesna) on Days 2–6 and cisplatin 20 mg/m^2 on Days 2–6. Cisplatin was administered with adequate pre- and post-hydration. Chemotherapy was suspended when leukopenia <3000/μl or thrombocytopenia <750 00/μl developed. Cisplatin was reduced to 75 and 50% if the estimated creatinine clearance was 45–60 ml/min and 30–45 ml/min, respectively. The regimen was repeated until disease progression, unacceptable toxicity, death or withdrawal of consent. We modified the interval of DIP regimen from 3 to 4 weeks due to the frequent hematological toxicity in our preliminary study.

The response was evaluated by computed tomography on Response Evaluation Criteria In Solid Tumors (RECIST) (17) every two therapy cycles, and toxicity was assessed according to Common Terminology Criteria for Adverse Events (CTCAE) (18).

The primary endpoints were progression-free survival (PFS) and overall survival (OS), calculated from the start of DIP chemotherapy. Secondary endpoints included objective response, safety and toxicity. Survival was estimated by the Kaplan–Meier method. We also examined the associations of various clinicopathologic factors with OS. Log-rank test and Cox proportional hazards model were used for univariate and multivariate analysis, respectively. All statistical analyses were carried out using JMP Pro 11.0.0 software (SAS Institute, Cary, NC, USA). A P value of 0.05 was considered significant.

Results

We enrolled 26 patients with histologically proven UC (Table 1), 22 men and 4 women, with a median age of 66 years [interquartile range (IQR): 56–71.25 years]. The primary sites were urinary bladder (n = 8), upper urinary tract (n = 14) and both (n = 4). The first-line regimens prior to enrollment were GC (n = 11), MVAC (n = 3) and both (n = 12), with a median of 6.0 treatment cycles (IQR: 4–7 cycles). Adjuvant chemotherapy had been performed in 13 cases (GC in

Table 1. Clinicopathologic characteristics of enrolled 26 patients

Parameter	Value
Age, years, median (IQR)	66 (56–71.25)
Sex, no. (%)	
Male	22 (85)
Female	4 (15)
ECOG performance status, no. (%)	
0	22 (85)
1	3 (12)
2	1 (4)
Primary site, no. (%)	
Bladder	8 (31)
Upper urinary tract	14 (54)
Both	4 (15)
Clinical T stage, no. (%)	
T ≤ 2	8 (31)
T ≥ 3	18 (69)
Number of metastatic organs, no. (%)	
1	9 (35)
2	13 (50)
3	4 (15)
Metastatic site, no. (%)	
Lymph node	19 (73)
Lung	12 (46)
Bone	7 (27)
Liver	5 (19)
Adrenal gland	2 (8)
Prior adjuvant chemotherapy, no. (%)	
Yes	13 (50)
No	13 (50)
Regimen(s) of prior salvage chemotherapy, no. (%)	
MVAC	3 (12)
GC	11 (42)
MVAC plus GC	12 (46)
Cycles of prior salvage chemotherapy, median (IQR)	6.0 (4–7)
Cause of introducing DIP chemotherapy, no. (%)	
Progression of disease	25 (96)
Adverse events	1 (4)
PFS, months, median (IQR)	3.0 (2–9.5)
OS, months, median (IQR)	8.5 (6.5–18.75)

IQR, interquartile range; MVAC, methotrexate/vinblastine/doxorubicin/cisplatin; GC, gemcitabine/cisplatin; DIP, docetaxel/ifosfamide/cisplatin; PFS, progression-free survival; OS, overall survival.

four and MVAC in nine) before enrollment, which were not counted as a first-line chemotherapy in this study.

Salvage DIP chemotherapy was indicated because of progression of disease ($n = 25$) or adverse events of prior chemotherapy ($n = 1$). Metastatic sites included lymph node ($n = 19$), lung ($n = 12$), bone ($n = 7$), liver ($n = 5$) and adrenal ($n = 2$), with a median of 2.0 metastatic organs (IQR: 1–2).

Table 2. Summary of objective responses of DIP chemotherapy

Maximal response	No. of patients (%)	Median duration of maximal response, months (IQR)
CR	1 (4)	31
PR	6 (23)	7 (5.5–14.5)
SD	6 (23)	11.5 (1.75–26.25)
PD	13 (50)	2 (1.5–3)

CR, complete response; PR, partial response; SD, stable disease; PD, progression disease.

Table 3. Summary of toxicities of DIP chemotherapy, according to CTCAE

Parameter	Grade 3/4, no. (%)	All grades, no. (%)
Hematotoxicity		
Leukopenia	20 (77)	23 (88)
Anemia	14 (54)	23 (88)
Febrile neutropenia	8 (31)	8 (31)
Thrombocytopenia	12 (46)	22 (85)
Diarrhea	3 (12)	3 (12)
Increased creatinine	0 (0)	4 (15)
Dyspnea	1 (4)	1 (4)
Ileus	1 (4)	1 (4)
Dysgeusia	0 (0)	1 (4)

A total of 96 cycles of DIP chemotherapy were administered across the 26 patients, with a median of 3.0 cycles per patient (IQR: 2–5 cycles). The median hospitalization period for one DIP regimen was 22 days (IQR: 16.95–25.5 days). The median PFS and OS was 3.0 months (IQR: 2–9.5 months) and 8.5 months (IQR: 6.5–18.75 months), respectively.

With regard to the secondary endpoints, one patient (4%) achieved complete response (CR) and six (23%) partial response (PR), while six (23%) attained stable disease (SD) (Table 2). The median duration of CR, PR and SD was 31, 7 and 12 months, respectively. Adverse events were leukocytopenia with CTCAE Grade 3 or 4 (77%), resulting in febrile neutropenia (31%) and Grade 3 or 4 thrombocytopenia (46%). Non-hematologic toxicities included diarrhea (11.5%), ileus (4%) and dyspnea (4%) (Table 3). There was no toxicity-related death.

In addition, univariate analysis showed that unresected primary lesion, multiple metastatic sites, no adjuvant chemotherapy and elevated lactate dehydrogenase were associated with poor OS. Multivariate analysis demonstrated multiple organ metastasis to be an independent poor prognostic factor (HR = 3.15, $P = 0.0364$) (Table 4).

Discussion

In the present study, we prospectively evaluated the efficacy and safety of DIP chemotherapy as ensuing salvage treatment after failure of standard cisplatin-based regimens for metastatic UC. Briefly, enrolled 26 patients received a median of 3.0 cycles of DIP chemotherapy, resulting in a median PFS of 3 months and a median OS of 8.5 months, respectively. Seven (27%) achieved major responses, with one CR (4%) and six PRs (23%).Grade 3/4 toxicities were mainly hematologic events and no toxicity-related death was observed.

Single chemotherapeutic and molecular-targeted agents show poor or no activity in Phase II trials in the second-line setting, with a few yielding only modest response rates of 10–20%, median PFS of 2–3

Table 4. Results of univariate and multivariate analyses assessing the association of various clinicopathologic factors with OS from the start of DIP chemotherapy

Variable	Cutoff	Univariate	Multivariate	
		P value	HR (95% CI)	*P* value
Sex	Male vs. female	0.0918		
Age, years	≥66 vs. <66[a]	0.2928		
ECOG performance status	≥2 vs. ≤1	0.0919		
Primary site	Bladder vs. others	0.9123		
Resection of primary site	No vs. yes	0.0235[b]	1.73 (0.213–10.9)	0.5749
Clinical T stage	≥3 vs. ≤2	0.0809		
Number of metastatic organs	≥2 vs. 1	0.0153[b]	3.15 (1.07–11.8)	0.0364[b]
Lymph node metastasis	Yes vs. no	0.5063		
Lung metastasis	Yes vs. no	0.7434		
Bone metastasis	Yes vs. no	0.4408		
Liver metastasis	Yes vs. no	0.2193		
Prior adjuvant chemotherapy	Yes vs. no	0.0438[b]	1.96 (0.787–4.97)	0.1468
Lines of prior salvage chemotherapy	2 vs. 1	0.8933		
Leukocyte counts, cells/μl	≥ vs. <ULN	0.4801		
Hemoglobin, g/dl	< vs. ≥LLN	0.3405		
Lactate dehydrogenase, IU/l	≥ULN vs. <ULN	0.0113[b]	2.14 (0.677–5.83)	0.1805
C-reactive protein, mg/dl	≥ULN vs. <ULN	0.3634		

HR, Hazard ratio; CI, confidential interval, ULN, upper limit of normal; LLN, lower limit of normal.

[a]Median.

[b]Statistically significant.

months and median OS of 6–9 months (8). Phase II and/or III trials of vinflunine (antitubulin), pemetrexed (antifolate), ixabepilone (nontaxane tubulin) and oxaliplatin (a third-generation platinum), as second-line treatment among cisplatin-pretreated patients (neoadjuvant/adjuvant cases being counted in some studies), resulted in 6–27.7% response rates, 1.5–2.9 months of PFS and 6.6–9.6 months of OS (8,19).

On the other hand, several multidrug combinations have been demonstrated to be effective as second-line treatments. Sternberg et al. (20) conducted a prospective study on an every-2-week regimen of gemcitabine (2500–3000 mg/m^2) and paclitaxel (150 mg/m^2) for advanced UC who had received prior cisplatin-based chemotherapy. Of 15 cases with prior salvage MVAC for metastatic disease, four (26.7%) achieved CR or PR and their PFS was 8.0 months. Suyama et al. (21) administered a combination of paclitaxel (180 mg/m^2 on Day 1) and gemcitabine (1000 mg/m^2 on Days 1, 8 and 15) for advanced or metastatic UC that had failed prior platinum-based chemotherapy. They showed that 10 out of 30 patients (33.3%) achieved either CR or PR and the median OS was 11.3 months. The present study yielded response rate and OS comparable with these previous reports on second-line chemotherapy.

Other promising multidrug chemotherapy regimens include combinations of gemcitabine, ifosfamide and cisplatin (response rate: 40.8%, OS: 9.5 months) (22) or paclitaxel, ifosfamide and nedaplatin (response rate: 40.0%, OS: 8.9 months) (23). However, these studies included patients who had received adjuvant or neoadjuvant therapy alone as the candidates, thus the efficacy of these regimens for metastatic UC that had failed salvage chemotherapy remained unknown. In our preliminary study, 9 of 14 patients underwent DIP as first-line treatment and the rest of 5 patients received it as second-line regimen after first-line MVAC chemotherapy. Our preliminary Phase 1 study was conducted by a small number of patients with better performance status. The present Phase 2 study included only cases unsuccessfully treated with at least one cisplatin-based regimen. We consider that this difference of background might result in disparity in efficacy.

With regard to prognostic factors for metastatic UC, there have been several reports mainly in the context of first-line salvage chemotherapy. Bajorin et al. (24) demonstrated that Karnofsky performance status (KPS) <80% and visceral metastasis (lung, liver or bone) were independent prognostic factors. Similarly, von der Maase et al. (4) showed that KPS < 80%, the presence of visceral metastasis, elevated alkaline phosphatase and the number of disease sites (>3) were significant prognostic parameters in patients with locally advanced or metastatic UC treated with GC or MVAC. Furthermore, we have recently reported that liver metastasis, poor performance status and higher leukocyte counts were independent poor prognostic factors for metastatic UC (25). On the other hand, studies concerning prognostic factors after failure of first-line salvage treatment are lacking. Sonpavde et al. (26) demonstrated performance status, hemoglobin, liver metastasis and time from prior chemotherapy to be poor prognostic factors from a retrospective review of 570 patients with metastatic UC failing in the first-line salvage chemotherapy. In the present study, we detected multiple organ metastases as an independent poor prognostic factor. Although this DIP regimen is intended to palliation and prolong life time, hospitalization period required for one DIP cycle is >3 weeks. Therefore, we have to make an effort to shorten hospitalization period, thereby utilizing outpatient-based maintenance chemotherapy.

Lastly, we had to admit that our study is to be cautiously interpreted due to a small sample size. Despite this, the results indicate moderate efficacy of DIP chemotherapy for metastatic UC that have failed standard cisplatin-based salvage regimens. A prospective investigation with a larger population under a longer follow-up period is needed to validate our observations.

Conflict of interest statement

None declared.

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BMC Urology

RESEARCH ARTICLE Open Access

Ultra-early versus early salvage androgen deprivation therapy for post-prostatectomy biochemical recurrence in pT2-4N0M0 prostate cancer

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Abstract

Background: The optimal timing of salvage androgen deprivation therapy (ADT) for biochemical recurrence after radical prostatectomy is controversial. We compared the outcomes of ultra-early versus early salvage ADT.

Methods: Among 855 patients undergoing radical prostatectomy at our institution between 2000 and 2012, we identified 121 with adjuvant-treatment-naïve pT2-4N0M0 prostate cancer who received salvage ADT for biochemical recurrence. These patients were divided into an ultra-early salvage ADT group (n = 51), who started salvage ADT before meeting the standardized definition of biochemical recurrence in Japan (two consecutive prostate-specific antigen [PSA] values ≥0.2 ng/ml), and an early salvage ADT group (n = 70) who started salvage ADT when they met the definition. The ultra-early ADT group consisted of those who started salvage ADT with a single PSA value ≥0.2 ng/ml (n = 30) or with two consecutive PSA values >0.1 ng/ml and rising (n = 21). The primary endpoint was biochemical recurrence after salvage ADT, defined as a single PSA value ≥0.2 ng/ml after PSA nadir following salvage ADT. Secondary endpoints were clinical metastasis and cancer-specific survival. A Cox proportional hazards model was used for multivariate analysis. The median follow-up was 65.5 months.

Results: Biochemical recurrence occurred in one patient (2.0%) in the ultra-early group and in 12 (17.1%) in the early salvage ADT group. Multivariate analysis identified ultra-early salvage ADT and preoperative Gleason score ≤7 as independent negative predictors of biochemical recurrence after salvage ADT. Only one patient in the early salvage ADT group developed clinical metastasis to a left supraclavicular lymph node, and no patient died from prostate cancer during follow-up. The major limitations of this study were its retrospective design, selection bias, and the possibility that the ultra-early salvage ADT group may have included patients without biochemical recurrence.

Conclusions: Ultra-early salvage ADT was an independent negative predictor of biochemical recurrence after salvage ADT in post-prostatectomy patients. Further consideration should be given to the use of salvage ADT before meeting the current definition of biochemical recurrence.

Keywords: Androgen deprivation therapy, Biochemical recurrence, Prostate cancer, Radical prostatectomy, Salvage androgen deprivation therapy

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Background

Approximately 25–35% of patients develop evidence of biochemical recurrence after radical prostatectomy for clinically localized prostate cancer [1,2]. Although salvage androgen deprivation therapy (ADT) is a popular option for the management of biochemical recurrence, uncertainty remains regarding which patients benefit from early salvage ADT and the ideal time at which to initiate therapy [3]. A retrospective analysis of 1,740 patients who underwent radical prostatectomy between 1990 and 1999 found no difference in systemic progression or cancer-specific survival between men who started salvage ADT at a prostate-specific antigen (PSA) level of ≥0.4 ng/ml compared with those who did not receive salvage ADT [4]. According to a similar analysis of 1,352 patients who underwent radical prostatectomy between 1988 and 2002, early salvage ADT for biochemical recurrence after radical prostatectomy was an independent predictor of delayed clinical metastases in high-risk cases, but not in the overall cohort [5].

However, these studies were mainly conducted before the era of ultrasensitive PSA assays, since when the definition of biochemical recurrence has changed. For example, Mir et al. recently advocated definitions of biochemical recurrence as any PSA ≥0.05 ng/ml in patients with nomogram-predicted 5-year progression-free probabilities of <50% [6].

We therefore compared the outcomes of early and very early administration of salvage ADT in patients treated with radical prostatectomy for localized prostate cancer.

Methods

We retrospectively reviewed 855 patients who underwent radical prostatectomy at our institution between 2000 and 2012 and identified 121 patients with adjuvant-treatment-naïve localized (pT2-4N0M0) prostate cancer who subsequently received continuous salvage ADT because of biochemical recurrence. Patients who underwent radiotherapy prior to or concomitant with hormonal therapy and patients who received any neo-adjuvant therapy were excluded from the study. The included patients were further divided into two groups: an ultra-early salvage ADT group (n = 51), in which patients started salvage ADT before meeting the standardized definition of post-prostatectomy biochemical recurrence in Japan (two consecutive PSA values ≥0.2 ng/ml [7]); and an early salvage ADT group (n = 70), in which patients started salvage ADT when they met the definition. The ultra-early salvage ADT group consisted of those who started salvage ADT with a single PSA value ≥0.2 ng/ml (n = 30) or with two consecutive PSA values >0.1 ng/ml and rising (n = 21). Patient allocation was not randomized. Doctors generally recommended early salvage ADT according to the General Rule for Clinical and Pathological Studies on Prostate Cancer of the Japanese Urological Association [8], but some patients were anxious and chose to have ultra-early salvage ADT. The clinicopathologic backgrounds of the two groups were similar, and the median durations of salvage ADT were 41 months in the ultra-early and 46 months in the early salvage ADT groups, respectively (Table 1). Patients who discontinued salvage ADT were treated as censored at the point of discontinuation. The median follow-up for all patients was 65.5 months (interquartile range [IQR]: 46–90.5 months) after radical prostatectomy, and an average of 33 PSA values were available per patient (i.e. total of 3,957 PSA values). We began using ultrasensitive PSA assays in September 2003.

The primary endpoint was biochemical recurrence after salvage ADT, defined as a confirmed single PSA value ≥0.2 ng/ml after PSA nadir following salvage ADT. Secondary endpoints were clinical metastasis and cancer-specific survival. Univariate analysis was conducted using the log-rank test and multivariate analysis was performed using the Cox proportional hazards model. All statistical analyses were carried out using JMP version 9.0.2 (SAS Institute, Cary, NC, USA). A value of $p < 0.05$ was considered significant.

This study was approved by the Ethics Committee, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo. Written informed consent for participation in this study was obtained from all participants.

As of 2014, the standardized definition of biochemical recurrence after radical prostatectomy in Japan was two consecutive PSA values ≥0.2 ng/ml at intervals of 2–4 weeks, as stated above [7], which is one of six standard definitions that have been used in published studies or are in current clinical use [6].

Results

One of 51 (2.0%) patients in the ultra-early salvage ADT group developed biochemical recurrence, compared with 12 of 70 (17.1%) patients in the early salvage ADT group (Figure 1). Univariate analysis demonstrated that timing of treatment (ultra-early salvage ADT versus early salvage ADT, p = 0.0279), preoperative PSA value (<20 ng/ml versus ≥20 ng/ml, p = 0.0493), and pathologic Gleason score (≤7 versus ≥8, p = 0.0043) were associated with the risk of biochemical recurrence after salvage ADT (Table 2).

Multivariate analysis identified ultra-early salvage ADT (p = 0.0118) and preoperative Gleason score ≤7 (p = 0.0182) as independent negative predictors of biochemical recurrence after salvage ADT (Table 3).

Regarding secondary endpoints, only one patient from the early salvage ADT group developed clinical metastasis to a left supraclavicular lymph node (p = 0.4233, log-

Table 1 Clinicopathologic characteristics of patients treated with ultra-early or early salvage ADT

Variable	Ultra-early salvage ADT (n = 51)	Early salvage ADT (n = 70)	p-value
Median age at prostatectomy, yr (IQR)	65 (61 - 69)	67 (60.75 - 72.25)	0.2992
Age at prostatectomy, no. (%):			0.0804
<70 yr	39 (76.5)	43 (61.4)	
≥70 yr	12 (23.5)	27 (38.6)	
Median preoperative PSA, ng/ml (IQR)	10.65 (7.3 - 15.56)	9.55 (6.3 - 12.07)	0.1903
Preoperative PSA, no (%):			0.6041
<20 ng/ml	46 (90.2)	61 (87.1)	
≥20 ng/ml	5 (9.8)	9 (12.9)	
Pathologic Gleason score, no. (%):			0.3256
≤6	12 (23.5)	23 (32.9)	
7	28 (54.9)	38 (54.3)	
8-10	11 (21.6)	9 (12.9)	
Pathologic tumor stage, no. (%):			0.8619
T2	32 (62.7)	45 (64.3)	
T3/4	19 (37.3)	25 (35.7)	
Extraprostatic extension, no. (%)	17 (33.3)	25 (35.7)	0.7859
Lymphovascular invasion, no. (%)	13 (25.5)	16 (22.9)	0.7376
Positive resection margin, no. (%)	37 (72.5)	43 (61.4)	0.2019
Seminal vesicle invasion, no. (%)	2 (3.9)	2 (2.9)	0.7464
Perineural invasion, no. (%)	35 (68.6)	43 (61.4)	0.4139
Median duration time of salvage ADT, months (IQR)	41 (22 - 66)	46 (27 - 73.25)	0.1326

ADT, androgen deprivation therapy; PSA, prostate-specific antigen; IQR, interquartile range.

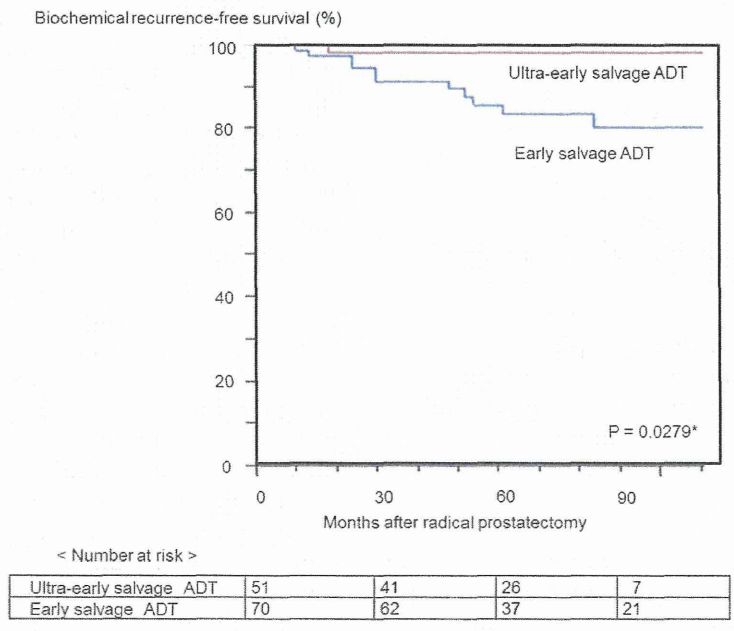

Figure 1 Kaplan-Meier curve depicting biochemical recurrence-free survival in patients treated with ultra-early versus early salvage ADT.

Table 2 Univariate analysis of the impact of various clinicopathologic factors on the risk of biochemical recurrence after salvage ADT

	No. of patients	p-value
Treatment group		0.0279*
Ultra-early salvage ADT	51	
Early salvage ADT	70	
Age, years		0.0798
<70	82	
≥70	39	
Preoperative PSA, ng/ml		0.0493*
<20	107	
≥20	14	
Pathologic Gleason score		0.0043*
≤7	101	
≥8	20	
Pathologic tumor stage		0.5690
≤2	77	
≥3	44	
Extraprostatic extension		0.7106
0	79	
1	42	
Lymphovascular invasion		0.4606
0	92	
1	29	
Positive resection margin		0.4467
0	41	
1	80	
Seminal vesicle invasion		0.3978
0	117	
1	4	
Perineural invasion		0.5035
0	43	
1	78	

ADT, androgen deprivation therapy; *statistically significant; PSA, prostate-specific antigen.

Table 3 Multivariate Cox proportional hazards regression analysis evaluating the impact of various clinicopathologic factors on the risk of biochemical recurrence after salvage ADT

	HR (95% CI)	p-value
Treatment group		
Ultra-early salvage ADT	Reference	
Early salvage ADT	7.691 (1.466-141.6)	0.0118*
Preoperative PSA, ng/ml		
<20	Reference	
≥20	2.065 (0.529-6.761)	0.2744
Pathologic Gleason score		
≤7	Reference	
≥8	4.739 (1.330-15.73)	0.0182*

ADT, androgen deprivation therapy; *statistically significant; PSA, prostate-specific antigen.

rank test), and no patient died from prostate cancer during the follow-up period. We were therefore unable to detect any differences in secondary endpoints between the two groups. This might be because the follow-up period was not long enough.

Discussion

ADT is a well-established treatment modality for patients with advanced prostate cancer. However, despite its proven efficacy in improving the quality of life in patients with metastatic disease, there is currently no consensus regarding the optimal timing of ADT after definitive local therapy [9]. Although a small randomized trial has supported the use of adjuvant ADT after radical prostatectomy in the setting of lymph node metastases [10,11], no randomized study has yet evaluated the utility of ADT for failure after radical prostatectomy, especially in patients without lymph node metastases [9].

Previous retrospective studies found no effect of salvage ADT on systemic progression-free survival or cancer-specific survival. Siddiqui et al. reviewed 1,740 patients who underwent radical prostatectomy between 1990 and 1999 and compared various PSA thresholds in relation to the timing of salvage ADT administration. They found no advantage in terms of systemic progression-free survival or cancer-specific survival in men who started salvage ADT at a PSA of 0.4, 1.0, or 2.0 ng/ml compared with those who did not receive salvage ADT [4]. A similar analysis by Moul et al. of 1,352 patients who underwent radical prostatectomy between 1988 and 2002 found that early salvage ADT for biochemical recurrence after radical prostatectomy was an independent predictor of delayed clinical metastases in high-risk patients (Gleason score ≥8 or PSA-doubling time ≤12 months), but had no impact on clinical metastases in the overall cohort [5].

However, these studies were mainly conducted before the era of ultrasensitive PSA assays. Ultrasensitive PSA assays allow a more precise measurement of ultrasensitive PSA values, possibly resulting in an optimal definition of biochemical recurrence after radical prostatectomy. For example, Mir et al. recently advocated the use of biochemical recurrence defined as any PSA ≥0.05 ng/ml in patients with nomogram-predicted 5-year progression-free probabilities of <50%, who might thereby benefit from early salvage radiotherapy [6]. While radiotherapy has been established as the most common salvage treatment for recurrence after radical prostatectomy in Europe and the

United States, ADT still plays an important role as salvage treatment in Asia. The Asia Consensus Statement 2013 in the NCCN Clinical Practice Guidelines in Prostate Cancer states that androgen deprivation is a candidate treatment option for post-radical prostatectomy recurrence in Asian patients negative for distant metastasis [12]. Indeed, radical prostatectomy and immediate adjuvant androgen deprivation therapy achieved excellent results, including a 10-year cancer-specific survival rate of 96.3% and 10-year estimated overall survival rate of 85.7% in Japanese patients with pT3N0 prostate cancer after a median follow-up period of 8.2 years [13]. Nevertheless, studies concerning the optimal timing of salvage ADT in the era of ultrasensitive PSA assays are lacking.

In the present study, patients who started salvage ADT before meeting a currently accepted definition of biochemical recurrence were less likely to develop subsequent biochemical recurrence after salvage ADT than those who started salvage ADT when they met the definition. By its very nature, ADT is less likely to contribute to local control than radiotherapy. However, ADT may improve local control when the residual tumor burden is very small, and adjuvant ADT after radical prostatectomy improved the clinical outcome in patients with adverse pathologic findings [10,11]. In a randomized prospective controlled trial, Messing et al. demonstrated that adjuvant ADT benefited patients with nodal metastases who had undergone prostatectomy and lymphadenectomy, compared with those who receive deferred treatment [10,11]. Referring to the results of European Organization for Research and Treatment of Cancer (EORTC) trial 30846, which found no benefit of immediate ADT compared with deferred therapy in men without definitive local treatment of the primary tumor [14], Messing et al. concluded that ADT was probably most effective against very small tumors [10,11,15]. The estimated tumor burden in EORTC 30846 was ≥12.5 cm^3 of cancer tissue, which was the mean local-tumor volume in Messing et al.'s patients [11]. Several experimental systems have provided collateral evidence to support the benefit of early ADT in inhibiting tumorigenesis [16-18].

A similar concept was recently demonstrated in the setting of salvage radiotherapy. Briganti et al. reported that timely administration of early salvage radiotherapy (given at PSA ≤0.5 ng/ml) was comparable to adjuvant radiotherapy for improving biochemical recurrence-free survival in pT3N0 prostate cancer, and suggested that initial observation followed by early salvage radiotherapy delivered at low PSA levels might be a viable option for the majority of surgically-managed patients with pT3N0 prostate cancer [19]. The present study may imply that we should consider using a PSA threshold below the currently accepted definition of biochemical recurrence in order to maximize the benefit from salvage ADT. Together with an excellent result of adjuvant ADT post-prostatectomy against pT3N0 prostate cancer [13], ADT might be more efficacious than other treatments in small tumors. However, ADT is associated with some real risks related to metabolic syndrome.

Our study had several limitations. The ultra-early salvage ADT group may have included patients without evidence of biochemical recurrence, which may have resulted in overestimation of the outcomes of ultra-early salvage ADT. Other limitations were its retrospective design, selection bias, lead-time bias, small sample size, and the inclusion of only Japanese subjects. Randomized prospective studies with longer follow-up periods are thus needed to confirm the benefits of ultra-early salvage ADT.

Conclusions

Ultra-early salvage ADT is an independent negative predictor of biochemical recurrence after salvage ADT in adjuvant-treatment-naïve pT2-4 pN0 M0 radical prostatectomy patients. The currently accepted definition of biochemical recurrence should be challenged in relation to the optimal timing of initiating salvage ADT.

Abbreviations

ADT: Androgen deprivation therapy; PSA: Prostate-specific antigen; IQR: Interquartile range; EORTC: European Organization for Research and Treatment of Cancer.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

ST made substantial contributions to study conception and design, data analysis and interpretation, and was involved in drafting the manuscript. HF made substantial contributions to study conception and design, data analysis and interpretation, and was involved in revising the manuscript critically for important intellectual content. TA, MS, TF, TN, AI, HK, and YI made substantial contributions to data acquisition. YH supervised the study, helped to draft the manuscript and was involved in revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Granulocyte macrophage colony-stimulating factor as a predictor of the response of metastatic renal cell carcinoma to tyrosine kinase inhibitor therapy

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Abstract. This prospective study was conducted to identify predictive markers for the response of metastatic renal cell carcinoma (RCC) to tyrosine kinase inhibitors (TKIs). Patients with histologically proven RCC with at least one measurable metastatic lesion were enrolled in this study. Blood samples were collected prior to treatment and the plasma levels of 27 cytokines were measured. Tumor response was assessed 8-12 weeks after the initiation of TKI treatment. A total of 13 patients (11 men and 2 women) with a median age of 63 years received sunitinib (8 cases), sorafenib (1 case), or axitinib (4 cases). Partial response (PR) was achieved in 5 patients (38%), stable disease (SD) in 4 (30%) and progressive disease (PD) was noted in 4 (30%). The plasma granulocyte macrophage colony-stimulating factor (GM-CSF) level in PR cases was significantly higher compared to that in SD or PD cases (P=0.012). Therefore, GM-CSF may be a predictive biomarker of the response of RCC to TKI treatment, suggesting that TKIs may exert clinical effects not only through suppression of the vascular endothelial growth factor, but also through immune system modulation.

Introduction

Renal cell carcinoma (RCC) is one of the major causes of cancer-related mortality. There were an estimated ~64,700 new cases of RCC and 13,570 deaths in 2012 in the United States (1). Over the last few years, a number of tyrosine kinase inhibitors (TKIs) have been proven to be effective and are currently widely used for the treatment of metastatic RCC. However, the effect of these TKIs appears to be rather limited, with only 31% of naive cases exhibiting an objective response [complete response (CR) or partial response (PR)] to sunitinib treatment in the first-line setting (2) and only 10% of cases with previous cytokine therapy exhibiting a PR to treatment with sorafenib (3). However, thus far, only a limited number of factors that predict the response of RCC to TKIs have been reported. A significant decrease in serum vascular endothelial growth factor (VEGF) receptor-2 levels and/or an increase in serum VEGF levels were observed in patients exhibiting an objective tumor response (4,5). Hypothyroidism and hypertension associated with TKI treatment were also reported to be correlated with a favorable response (6,7).

Although previous studies suggested that TKIs may affect the immune system (8,9), only a limited number of studies have investigated immunological biomarkers for therapeutic prediction. Adotevi *et al* (10) reported that a decrease in regulatory T cells was correlated with a favorable overall survival in cases with metastatic RCC who received sunitinib-based antiangiogenic therapy. Thus, we conducted a prospective study to invesigate predictive immunological biomarkers.

Patients and methods

Patients. Patients with histologically proven RCC with at least one measurable metastatic lesion, who were diagnosed between March, 2012 and June, 2013, were enrolled in this study. Sunitinib, sorafenib or axitinib were administered orally as previously described (2,3,11). Tumor response was assessed 8-12 weeks after the initiation of TKI treatment according to the response evaluation criteria in solid tumors and was classified as CR, PR, stable disease (SD) or progressive disease (PD) (12).

We collected blood samples from the 13 patients prior to treatment. The plasma was deep frozen at -80°C and stored before measuring the immune function.

Cytokines. A total of 27 cytokines including interleukin (IL)-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9,

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Key words: metastatic, renal cell carcinoma, tyrosine kinase inhibitors, granulocyte macrophage colony-stimulating factor

Table I. Correlation between the clinical effect of tyrosine kinase inhibitors (TKIs) and clinicopathological characteristics among patients with metastatic renal cancer.

Clinical characteristics	Total (n=13)	Clinical effect[a] PR (n=5)	SD (n=4)	PD (n=4)	P-value
Gender					
Male	11	4	4	3	0.603
Female	2	1	0	1	
Age (years)					
≥65	7	2	2	3	0.593
<65	6	3	2	1	
Performance status					
0	8	3	2	3	0.780
1	5	2	2	1	
Laterality					
Right	8	2	3	3	0.479
Left	5	3	1	1	
Nephrectomy					
Radical	11	4	4	3	0.603
Partial	2	1	0	1	
Histology					
Clear cell RCC	11	5	2	4	0.085
Papillary RCC	2	0	2	0	
Nuclear grade					
G1/G2	12	4	4	4	0.449
G3	1	1	0	0	
Stage					
pT1	6	3	1	2	0.593
pT2/pT3/pT4	7	2	3	2	
Lymphovascular invasion					
0	2	1	1	0	0.603
1	11	4	3	4	
Lung metastasis					
No	3	1	2	0	0.267
Yes	10	4	2	4	
Bone metastasis					
No	8	2	4	2	0.180
Yes	5	3	0	2	
TKIs					
Sunitinib	8	4	3	1	0.219
Others	5	1	1	3	
Dose intensity (%)					
100	7	2	2	3	0.593
<100	6	3	2	1	
Previous treatment					
No	2	1	1	0	0.603
Yes	11	4	3	4	
Previous TKI treatment					
No	8	4	3	1	0.219
Yes	5	1	1	3	
Previous cytokine treatment					
No	5	3	1	1	0.479
Yes	8	2	3	3	

Table I. Continued.

Clinical characteristics	Total (n=13)	Clinical effect[a] PR (n=5)	SD (n=4)	PD (n=4)	P-value
Previous mTOR inhibitor treatment					
No	10	4	3	3	0.980
Yes	3	1	1	1	

[a]Best response during the 3-month treatment. The P-values were calculated using the Kruskal-Wallis test. PR, partial response: SD, stable disease: PD, progressive disease: RCC, renal cell carcinoma: mTOR, mammalian target of rapamycin.

IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), IFN-γ-induced protein 10, monocyte chemoattractant protein-1, macrophage inflammatory protein (MIP)-1α, platelet-derived growth factor (PDGF)-BB, MIP-1β, regulated on activation, normal T-cell expressed and secreted, tumor necrosis factor-α and VEGF were measured twice by BioPlex Pro Human Cytokine 27 Plex assay (M50-0KCAF0Y; Bio-Rad, Hercules, CA, USA). The assay was performed according to the manufacturer's instructions. Briefly, plasma was centrifuged at 15,000 x g for 10 min at 4˚C. The samples were then incubated with microbeads labeled with specific antibodies to one of the aforementioned cytokines for 60 min. Following a washing step, the beads were incubated with the detection antibody cocktail, with each antibody specific to a single cytokine, for 30 min. After another washing step, the beads were incubated with streptavidin-phycoerythrin for 10 min, washed again and the concentration of each cytokine was determined using the array reader. The samples were tested in duplicate on a 96-well plate alongside the standard curve used to generate the results. Unknown concentrations were calculated from a standard curve generated from Bio-Rad supplied standards.

Statistical analysis. The correlation between clinical and cytokine data was analyzed by analysis of variance (ANOVA) and Tukey-Kramer's test using JMP software, version 10.0.0 (SAS, Institute, Cary, NC, USA).

This study was approved by the Institutional Ethics Committee of the Faculty of Medicine and Graduate School of Medicine of the University of Tokyo (no. H22-23-400).

Results

Patient characteristics. A total of 13 patients (8 treated with sunitinib, 1 with sorafenib and 4 with axitinib), including 11 men and 2 women, with a median age of 63 years (range, 50-77 years), were recruited in this study (Table I). The performance status was 0 in 8 and 1 in 5 cases. Eight tumors were located in the right and 5 in the left kidney. Radical nephrectomy was performed in 11 and partial nephrectomy in 2 patients. Histologically, the tumors were diagnosed as 11 clear cell RCCs and 2 papillary RCCs. All the patients had developed metastasis, with the most common metastatic site being the lung (10 cases), followed by bone (5 cases).

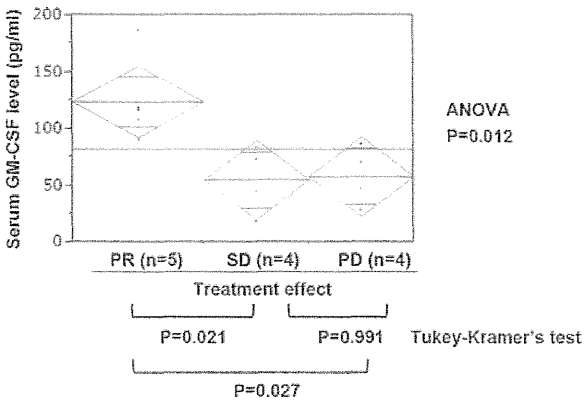

Figure 1. Comparison of serum granulocyte macrophage colony-stimulating factor (GM-CSF) levels among patients who achieved partial response (PR), stable disease (SD) or exhibited progressive disease (PD) after treatment with tyrosine kinase inhibitors. ANOVA, analysis of variance.

Treatment. Two cases received TKI treatment as first-line therapy. Previous systemic treatment included TKIs in 5, mammalian target of rapamycin (mTOR) inhibitors in 3 and cytokines in 8 patients. PR was achieved in 5 cases (38%), SD in 4 (30%) and PD developed in 4 cases (30%). The dose was reduced in 6 patients (46%) due to adverse events.

GM-CSF plasma levels by treatment response. No clinical parameters exhibited a significant correlation with treatment effect (Table I). Among the 27 investigated cytokines, the plasma GM-CSF level in PR cases was significantly higher compared to that in cases with SD or PD (Fig. 1, ANOVA, P=0.012; Tukey-Kramer's test: PR vs. SD, P=0.021; PR vs. PD, P=0.027; and SD vs. PD, P=0.991). The IL-6 level was higher in PD cases, but the difference was not statistically significant (Table II, P=0.141).

Discussion

We demonstrated that plasma GM-CSF may be a predictive marker of the response to TKI treatment. Thus far, only a few studies demonstrated the clinical utility of GM-CSF. The

Table II. Correlation between the clinical effect of tyrosine kinase inhibitors and cytokine levels in patients with metastatic renal cancer.

	Clinical effect			
Cytokines	PR	SD	PD	P-value
GM-CSF	123±36	54±29	57±25	0.012
IL-1β	3.8±4.6	1.9±1.2	1.6±0.2	0.494
IL-1ra	103±98	57±43	60±22	0.536
IL-2	5.2±2	4.8±3.1	5±3	0.971
IL-4	5.6±2.3	4.8±1.8	5.1±2.2	0.864
IL-5	1.1±1.4	0.7±0.8	0.6±0.8	0.791
IL-6	6±2.3	5±2.6	12±8.9	0.141
IL-7	5.3±1.9	4.8±4.9	3.1±2.2	0.605
IL-8	25±13	29±33	19±12	0.779
IL-9	44±12	26±8.8	29±15	0.125
IL-10	4.2±2.9	3.4±1.1	6.4±5.7	0.525
IL-12	19±17	17±15	32±37	0.658
IL-13	5.2±3.9	5.1±3.1	5.1±2.4	0.990
IL-15	5±1.6	3.6±2.3	4.2±0.6	0.479
IL-17	56±15	41±16	59±41	0.613
Eotaxin	183±152	128±126	112±61	0.667
FGF-basic	51±14	42±12	55±22	0.554
G-CSF	66±19	52±18	59±13	0.527
IFN-γ	610±893	207±94	178±21	0.462
IP-10	2,381±1,857	1,386±749	1,906±1,432	0.616
MCP-1	82±68	41±16	47±22	0.388
MIP-1α	2.9±1.1	7.7±12	2.9±1.7	0.561
PDGF-BB	309±306	862±146	213±128	0.508
MIP-1β	178±43	174±141	128±79	0.703
RANTES	3,364±138	2,630±763	2,679±771	0.523
TNF-α	88±92	62±47	43±6.9	0.580
VEGF	108±62	122±79	165±143	0.683

The results are expressed as mean ± standard deviation (pg/ml) and the P-values were calculated using analysis of variance. PR, partial response; SD, stable disease; PD, progressive disease; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; IFN-γ, interferon-γ; IP-10, IFN-γ-induced protein 10; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; RANTES, regulated on activation, normal T-cell expressed and secreted; PDGF, platelet-derived growth factor; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

plasma GM-CSF level was found to be higher in cervical cancer patients compared to healthy controls (13), while in another study GM-CSF was undetectable in non-cancer patients (14).

GM-CSF promotes the differentiation and expansion of myeloid-derived suppressor cells (MDSCs) (15,16). Antigen-specific $CD8^+$ T-cell tolerance, induced by MDSCs, is known to be one of the main mechanisms of tumor escape (17). Knockdown of GM-CSF in tumor cells may reverse the cytotoxicity to CD8 T lymphocytes: Dolcetti *et al* (15) found that lack of GM-CSF release from 4T1 mammary carcinoma cells reduced the accumulation of $Gr\text{-}1^{int/low}$ MDSC subsets and successfully inhibited tumor-induced tolerance in mice. Similarly, Serafini *et al* (16) demonstrated that inhibition of MDSC function abrogates the proliferation of regulatory T cells and tumor-induced tolerance in antigen-specific T cells, using the A20 B-cell lymphoma model *in vitro* and *in vivo*. However, TKIs may reduce the number of MDSCs in the tumor and normalize T-lymphocyte function: Xin *et al* (18) demonstrated that sunitinib directly induced RCC tumor cell apoptosis through Stat3 inhibition, which was accompanied by a reduction in MDSCs and tumor-infiltrating regulatory T cells.

These reports suggest that high levels of plasma GM-CSF may promote the function of MDSCs and escape of tumor cells from the host immune system. In patients with high GM-CSF levels, TKIs may decrease the function of MDSCs that is upregulated by GM-CSF and reverse the cytotoxicity of regulatory T lymphocytes directly or indirectly, which may lower tumor-induced tolerance and result in favorable treatment effects.

In our study, VEGF was not found to be significantly associated with treatment effect, contrary to previous reports (4,5). GM-CSF was reported to induce VEGF release from the epithelium, resulting in the promotion of carcinogenesis: Wang *et al* (19) demonstrated that, in a colitis-associated cancer model, blocking GM-CSF activity *in vivo* significantly decreased epithelial release of VEGF and abrogated cancer formation. In the plasma, GM-CSF, which is upstream of VEGF, may be a more sensitive biomarker for metastatic RCC treatment compared to VEGF.

As regards other biomarkers, Tran *et al* (20) screened pretreatment cytokines and angiogenic factors in patients with metastatic RCC who received pazopanib treatment and found that high IL-6 was predictive for unfavorable progression-free survival. In our study, IL-6 was also higher in PD cases, but the difference was not statistically significant.

This study had certain limitations. First, this was a single-institution study; and second, our sample size was limited.

In conclusion, high pre-treatment plasma levels of GM-CSF, which is an inducer of immune tolerance, were significantly associated with a favorable response of metastatic RCC to TKI treatment. The result suggests the potential of GM-CSF as a predictive biomarker of the response to TKI treatment. However, further investigation is required to determine the effects of TKIs on abrogating cancer immune tolerance.

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