In conclusion, this analysis suggests, to achieve better outcomes, some medical system interventions can be promising on the basis of a comprehensive regional palliative care program: (1) routine proactive care planning based on validated prognosis estimation, (2) reconstructing social resources to increase informal caregivers, (3) establishing formal community palliative care services as easily available and a 24-h 7-day service, (4) using a simple visible and routine-need assessment tool with clear instruction of when and how to consult palliative care services in the region, (5) ensuring enough time for each clinician to address patients' concerns, and (6) research to explore more effective palliative treatment of frequent but difficult symptoms.

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Impaired mental health among the bereaved spouses of cancer patients

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Abstract

Objective: **Few cancer physicians routinely provide bereavement follow-up in clinical practice. The purpose of this study was to identify the prevalence of impaired mental health among the bereaved spouses over several years and explore the indicators for early detection of high-risk spouses during end-of life (EOL) care.**

Methods: **A cross-sectional mail survey was conducted for the bereaved spouses of patients who had died at the National Cancer Center Hospital of Japan. Bereaved spouses with potential psychiatric disorders were identified by the cut-off score of the 28-item General Health Questionnaire. Associated factors of potential psychiatric disorders were explored by logistic regression analysis.**

Results: **A total of 821 spouses experiencing bereavement from 7 months to 7 years returned the questionnaires. Overall mean prevalence of potential psychiatric disorders was 44% (360/821). Bereaved spouses 'under 55 years' (71%) or '2 years after bereavement' (59%) revealed a significantly higher prevalence ($p < 0.01$). Associated factors during EOL care were several characteristics such as 'spouses' history of psychiatric disorder (odds ratio (OR) = 3.19), 'patients' with stomach cancer (OR = 1.87), and 'patients' using psychiatric consultation services (OR = 1.52) as well as spouses' dissatisfaction with EOL care such as 'physicians' treatment of physical symptoms' (OR = 3.44) and 'time spent communicating with patients' (OR = 1.55).**

Conclusions: **Nearly half the bereaved spouses showed potential psychiatric disorders even 7 years after bereavement. Patients' psychological distress, spouses' history of psychiatric disorder, and dissatisfaction with EOL care were indicators of high-risk spouses.**

Introduction

Conjugal bereavement was the strongest risk factor for depression among elderly community subjects in a meta-analysis of 20 studies (odds ratio (OR) = 3.3) [1] and bereaved spouses showed a significant increase in the risk of depression compared with married people in large cohort studies (1.5-fold, 3.6-fold) [2,3]. In oncology settings, spouses experienced the highest levels of distress among family members at the time of patient death [4] and bereavement brought an increased risk of major depressive disorder [5,6]. Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008 [7]; however, few cancer physicians routinely provide bereavement follow-up in clinical practice [8].

Several longitudinal studies have reported that impaired mental health among the bereaved clearly diminishes over time. The prevalence of major depressive disorder among caregivers of cancer patients was identified by clinical interview: 28% at the time of hospice enrollment, 12% at 6 months after death, and 7% at 1 year after death [5,6]. Depression, anxiety, and grief measured by self-administered questionnaire decreased during the first year after bereavement [9–11] and then remained unchanged over the next year [11]. On the other hand, cross-sectional studies reported that negative effects such as anger, sadness, self-blame, and guilt did not decrease among those who had been bereaved for more than 4 years [12,13] and 25% of the bereaved parents had not worked through their grief even 4–9 years after the loss [14]. However,

these persistent symptoms could not predict the prevalence of potential psychiatric disorders among the bereaved.

Impaired mental health among the bereaved who have lost a relative to cancer is associated with several characteristics of the patients and the bereaved. As for clinical characteristics of cancer patients, 'short duration of hospice enrollment' [5,6], 'intensive end-of-life (EOL) care' [15], and 'ICU death' [16] were associated with impaired mental health among the bereaved. In addition, bereaved characteristics of 'under 65 years' [9], 'female' [5,17,18], 'spouse' [5], 'prior physical symptoms' [5], 'prior depression' [5,9,17], and 'anticipatory grief' [16] were also reported. However, these associated factors are not useful as indicators for early detection of high-risk spouses during EOL care in clinical practice at a hospital even though 90% of cancer patients in Japan die in a hospital [19].

In the present study, the primary purpose was to identify the prevalence of impaired mental health that can be used to predict the prevalence of potential psychiatric disorders among the bereaved who have lost their spouse to cancer. The secondary purpose was to investigate associated factors of the prevalence so that we could suggest the indicators for early detection of high-risk spouses during EOL care.

Methods

Study sample

We conducted a cross-sectional mail survey for the bereaved spouses whose partner had died at the National Cancer Center Hospital East (NCCHE). This study was approved by the Institutional Review Board and Ethics Committee of the National Cancer Center of Japan in January 2009.

First, in January 2009, we found it necessary to identify family members to whom we intended to mail study participation invitations; this was because of a lack of accurate data about marital status in the hospital patient database. Eligibility criteria were (i) patient's primary clinician belonging to the eight divisions cooperating with this study (Hematology, Pancreatic, Head and Neck, Gastric Surgery, Gastrointestinal, Thoracic Surgery, Thoracic Oncology, and Palliative Care), which covered 98% of the patients who died at NCCHE; (ii) patient's data available in the hospital's patient database operating since January 2001; and (iii) patient's death occurring at least 6 months earlier. Exclusion criteria and flow of the study sample are explained in Figure 1.

We matched the demographic characteristics of the deceased cancer patients drawn from the hospital patient database with those of the bereaved spouses based on the completed questionnaires. Respondents' characteristics ($n=821$) showed a lower proportion of males (30%, $n=242$ vs. 36%, $n=753$, $p<0.01$) and a shorter duration of bereavement (3.0 ± 1.9 vs. 3.2 ± 2.0 years, $p<0.01$) compared with the non-responders ($n=2081$) among the 2902 candidate participants; the difference in values of the deceased patients' characteristics such as age, duration of last hospital admission, place of death, history of usage of psychiatric consultation services, and cancer site was not significant.

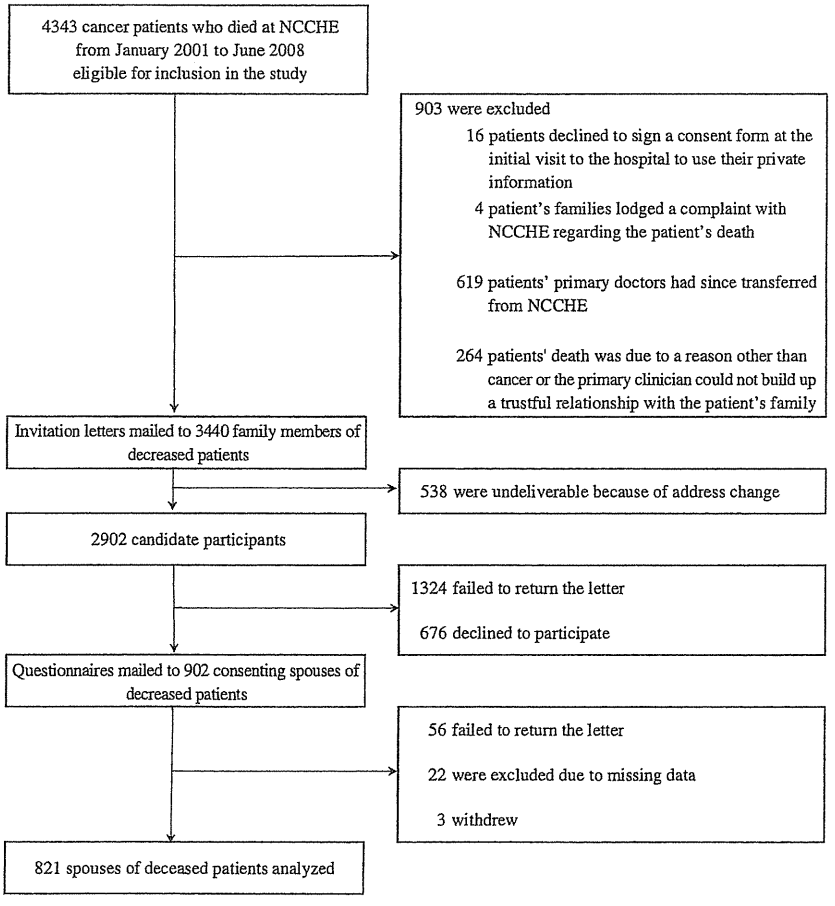

Figure 1. Flow of study sample

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Measures

Deceased patients' characteristics

We examined the overall computerized patient database of NCCHE to identify cancer patients' characteristics. Time since cancer diagnosis to death was declared in the questionnaires completed by the bereaved. History of usage of psychiatric consultation services was identified by using the consultation database developed by the Psychiatric Services Division of the NCCHE. This computerized database [20] includes demographic variables and psychiatric disorders of patients who were referred to the Psychiatric Services Division.

Bereaved spouses' characteristics

The questionnaires completed by the bereaved spouses included physical and psychological information such as physical illness under treatment and history of psychiatric disorder prior to their partner's death as well as demographic variables.

Dissatisfaction with EOL care

The bereaved spouses retrospectively reported their dissatisfaction with EOL caregiving (five items) and physician's EOL care (four items) during the month prior to the patient's death using a five-point Likert-type scale (0: very satisfied, 1: fairly satisfied, 2: neutral 3: fairly dissatisfied, 4: very dissatisfied). We rescored each item as 0 (absence of dissatisfaction, 0–2) or 1 (presence of dissatisfaction, 3–4) in this study.

Impaired mental health

The General Health Questionnaire (GHQ), using a four-point Likert-type scale (possible range, 0–3; higher scores indicate impaired mental health), has been widely used to detect persons with nonspecific psychiatric disorders [21]. We used the validated Japanese 28-item version (GHQ28 [22]). Persons with potential psychiatric disorders were identified by the cut-off score of the GHQ scoring method (0-0-1-1; possible range, 0–28; cut-off score, 5/6). This cut-off score showed the best sensitivity and specificity when compared with the ratings of the clinical interview [23,24] and this approach has shown its applicability to the Japanese version [22].

Statistical analysis

Impaired mental health was compared using analysis of variance with the Bonferroni multiple comparison method or *t*-test. Potential psychiatric disorders were compared by using the chi-square test with residual analysis. Variables showing p-values < 0.05 in the univariate analysis were entered as independent variables in a multivariate logistic regression analysis with backward elimination to identify associated factors of potential psychiatric disorders.

P-values < 0.05 were considered significant and all p-values were two-tailed. All statistical analyses were carried out using SPSS ver.12.0J for Windows (SPSS Japan Institute Inc., Tokyo, Japan).

Results

Characteristics of deceased patients/bereaved spouses

Table 1 summarizes the characteristics of the 821 participants experiencing bereavement from 7 months to 7 years.

Table 1. Characteristics of deceased patients and bereaved spouses ($n = 821$)

	Mean ± SD (median, range)	*n*	(%)
Deceased patients' characteristics			
Age, years	64 ± 9.0 (65, 32–88)		
Time since cancer diagnosis to death, months	27 ± 29 (16, 1–187)		
Duration of last hospital admission, days	27 ± 29 (17, 1–208)		
Bereaved spouses' characteristics			
Age, years	66 ± 9.0 (66, 32–89)		
Time since bereavement, years	3.0 ± 1.9 (3.0, 0.6–7.2)		
Gender			
Male		242	30
Female		579	70

SD, standard deviation.

In this study, 579 bereaved (70%) were female, 441 patients (54%) died in the Palliative Care Unit, and 629 bereaved (77%) were involved in EOL caregiving 'everyday'.

Prevalence of impaired mental health and potential psychiatric disorders

As shown in Table 2, we estimated the population of bereaved spouses to be 2649 by multiplying the total number of 4343 deceased patients by 0.61, which is the approximate ratio of Japanese cancer patients who have a spouse at the time of death among overall cancer deaths in Japan in 2007 (206,389/336,139)[19]. As a result, the overall sampling rate (estimated) was 31% (821/2,649), and the prevalence of potential psychiatric disorders was 44% (360/821, 95% CI = 40.6–47.4).

With impaired mental health, three-way interaction (age × gender × time) was not significant (F (18, 689) = 1.56, $p = 0.07$). Two-way interaction (age × gender: F (3, 689) = 2.75, $p = 0.04$) was significant: males 'under 55 years' showed significantly greater prevalence than males '55–64 years' or 'over 75 years' (F (3, 214) = 3.66, $p = 0.01$, A0 > A1, A3, $p < 0.05$) and females 'under 55 years' or '55–64 years' showed significantly greater prevalence than females '65–74 years' (F (3, 533) = 4.65, $p < 0.01$, A0, A1 > A2, $p < 0.05$). The main effect of time was significant (F (6, 689) = 2.71, $p = 0.01$): the bereaved who had lost their spouse '2 years ago' revealed significantly greater prevalence than those who had lost their spouse '4 years ago' with multiple comparison (F (2, 738) = 3.31, $p < 0.01$, T2 > T4, $p < 0.05$).

The prevalence of the bereaved varied with age and time: 'under 55 years' (71%) revealed significantly higher prevalence than those '65–74 years' (42%) (χ^2 (3) = 23.17, $p < 0.01$, A0 > A2, $p < 0.01$) and the bereaved who had lost their spouse '2 years ago' (59%) revealed significantly higher prevalence than those who had lost their spouse '4 years ago' (37%) (χ^2 (6) = 17.81, $p < 0.01$, T2 > T4, $p < 0.01$). No significant difference was observed between genders (χ^2 (1) = 1.08, $p = 0.34$).

Factors associated with potential psychiatric disorders

In the univariate analysis, 14 variables were significantly associated with potential psychiatric disorders ($p < 0.05$, Table 3). Table 4 shows the results of a multivariate logistic regression analysis: 'patients using psychiatric consultation

Table 2. Prevalence of impaired mental health and potential psychiatric disorders among bereaved spouses of cancer patients

			Deceased patients	Population[a] (estimated)	Sample	Sample rate (estimated)	Impaired mental health (GHQ28, 0–28)		Potential psychiatric disorders (GHQ28 ≥ 6)	
	Year	Group	*N*	*N'*	*n*	% (*n*/*N'*)	Mean (SD)	*n'*	% (*n'*/*n*)	95% CI
Total			4343	2649	821	31	7.17 (6.79)	360	44	40.6–47.4
Age										
	–54	A0			75		(9.95) 6.59	53	71	60.4–81.0
	55–64	A1			232		7.65 (6.77)	118	51	44.5–57.3
	65–74	A2			339		6.37 (6.68)	141	42	36.4–46.9
	75–	A3			109		6.62 (6.77)	46	42	32.9–51.5
Gender										
Male			1494	911	220	24	6.93 (6.65)	98	45	37.9–51.1
Female			2849	1738	538	31	7.27 (6.86)	262	49	44.5–52.9
Time since bereavement										
	<1	T0	258	157	55	35	8.67 (7.41)	30	55	41.3–67.7
	<2	T1	668	407	133	33	7.79 (7.38)	66	50	41.1–58.1
	<3	T2	611	373	134	36	8.60 (6.92)	79	59	50.7–67.3
	<4	T3	616	376	111	30	6.00 (6.29)	44	40	30.5–48.7
	<5	T4	643	392	96	24	5.48 (6.05)	35	37	26.9–46.1
	<6	T5	671	409	108	26	6.74 (6.56)	45	42	32.4–51.0
	≥6	T6	876	534	108	20	6.97 (6.55)	55	51	41.5–60.3

Some percentages do not add up to 100% because of missing data.
SD, standard deviation; CI, confidence interval.
[a]Population was estimated by multiplying the number of deceased patients (*N*) by 0.61, which is the approximate ratio of Japanese cancer patients who have a spouse at the time of death among overall cancer deaths in Japan in 2007.

services' (OR = 1.52), 'patients with stomach cancer' (OR = 1.87), and 'bereaved with a history of psychiatric disorder' (OR = 3.19) were significantly associated factors among the characteristics of patients/bereaved prior to the patient's death. Additionally, 'time spent communicating with patients' (OR = 1.55) and 'physician's treatment of physical symptoms' (OR = 3.44) were significantly associated factors among the bereaved spouses' dissatisfaction with EOL care during the final month.

Discussion

In this study, we identified a considerably high prevalence of potential psychiatric disorders among the bereaved (44% of total respondents). Patients' psychological distress, bereaved spouses' history of psychiatric disorder, and dissatisfaction with EOL care were indicators for early detection of high-risk spouses prior to the patient's death.

Our results indicated that, even 7 years after losing their spouse, a significant number of the bereaved have potential psychiatric disorders (37–59%). This is a higher prevalence than that of consecutive patients in general practice in Britain (35%) [25] and is three-fold higher than that of a healthy sample in Japan (14%) [22]. We discuss this high prevalence from two aspects of the results. First, more than half the spouses within less than 3 years since bereavement showed potential psychiatric disorders. This high prevalence might be inflated by normal grief, a common psychological reaction among the bereaved. Our results support those of the previous studies in which prevalence decreased during the first year after bereavement [9–11]. However, our results do not support previous results where prevalence remained unchanged over the second year [11]. This discrepancy might partly be because of spouses participating in the Japanese Buddhist rite of *sankaiki* where bereaved families gather together on the second anniversary of the death and reminisce about the deceased. This mourning ceremony might increase the psychological distress of the bereaved by triggering negative psychological states such as yearning, an unfulfilled desire to reunite with the deceased. Second, around 40% of the respondents whose bereavement was 3–7 years earlier showed potential psychiatric disorders. Even though their psychological distress might have eased somewhat after the mourning ceremony in the second year, the prevalence of both impaired mental health and potential psychiatric disorders was considerably high among the spouses after bereavement. This result could be because of subsequent physical problems of the bereaved because 'physical illness under treatment' was significantly associated with morbidity. However, this persistent prevalence might suggest prolonged bereavement distress because dissatisfaction with EOL (their caregiving and the physician's care) was strongly associated with potential psychiatric disorders in this study.

Among the characteristics of patients/bereaved, 'bereaved spouse's history of psychiatric disorders prior to the patient's death' was the most highly correlated factor (OR = 3.19) and replicated previous studies on the indicators of vulnerability to bereavement stress [5,9,17]. Patients with stomach cancer in this study might have a higher rate of psychological symptoms because the highest rate of mixed anxiety/depression symptoms (20%) was seen with stomach cancer patients among 22 cancer types in a large cohort study [26]. Considering the positive association between patient and caregiver psychological distress in meta-analyses [27,28], patients' psychological distress factors of 'stomach cancer' or 'usage of psychiatric consultation service' could raise spouses' psychological distress prior to the patient's death. In addition, because psychological distress of caregivers prior to the patient's death predicted its prevalence after bereavement in a longitudinal multisite study [16], the initial detection of spouses with high psychological distress prior to the patient's death might be the most useful strategy for preventing subsequent impaired mental health among the bereaved.

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Table 3. Factors associated with potential psychiatric disorders among bereaved spouses of cancer patients: univariate analysis

Variables	Total		Potential psychiatric disorders: Presence		Potential psychiatric disorders: Absence		Analysis	
	n	(%)	*n*	(%)	*n*	(%)	χ^2	*p*
Deceased patients' characteristics								
Age (< 65 years)	386	(47.0)	198	(51.3)	188	(48.7)	4.56	0.04
Time since cancer diagnosis to death (< 1 year)	285	(34.7)	144	(50.5)	141	(49.5)	1.69	0.20
Duration of last hospital admission (< 1 week)	182	(22.2)	93	(51.1)	89	(48.9)	1.25	0.27
Place of death (Palliative care unit)	402	(49.0)	190	(47.3)	212	(52.7)	0.02	0.94
History of usage of psychiatric consultation service	152	(18.5)	87	(57.2)	65	(42.8)	7.24	<0.01
Cancer site				(51.3)		(48.7)		
Lung	241	(29.4)	113	(46.9)	128	(53.1)	0.05	0.88
Pancreas	88	(10.7)	39	(44.3)	49	(55.7)	0.40	0.57
Stomach	60	(7.3)	38	(63.3)	22	(36.7)	6.56	0.02
Colon	63	(7.7)	24	(38.1)	39	(61.9)	2.42	0.15
Head and neck	60	(7.3)	25	(41.7)	35	(58.3)	0.89	0.42
Esophagus	45	(5.5)	26	(57.8)	19	(42.2)	2.03	0.17
Breast	41	(5.0)	20	(48.8)	21	(51.2)	0.03	0.87
Liver	38	(4.6)	17	(44.7)	21	(55.3)	0.12	0.74
Biliary tract	33	(4.0)	19	(57.6)	14	(42.4)	1.41	0.29
Lymphoma	9	(1.1)	4	(44.4)	5	(55.6)	0.03	1.00
Bereaved spouses' characteristics								
Age (< 65 years)	307	(37.4)	171	(55.7)	136	(44.3)	13.94	<0.01
Gender (Male)	220	(26.8)	98	(44.5)	122	(55.5)	1.08	0.34
Time since bereavement (< 3 years)	322	(39.2)	175	(54.3)	147	(45.7)	10.55	<0.01
Living status (Living alone)	363	(44.2)	171	(47.1)	192	(52.9)	0.04	0.88
Employment status (Employed)	216	(26.3)	106	(49.1)	110	(50.9)	0.30	0.63
Education (≤9 years)	121	(14.7)	51	(42.1)	70	(57.9)	1.65	0.23
Physical illness under treatment	424	(51.6)	227	(53.5)	197	(46.5)	14.10	<0.01
History of any psychiatric disorder prior to patients' death	60	(7.3)	43	(71.7)	17	(28.3)	15.37	<0.01
Bereavement experience after the death of spouse	196	(23.9)	91	(46.4)	105	(53.6)	0.12	0.74
Religiousness	311	(37.9)	157	(50.5)	154	(49.5)	1.89	0.18
Involvement in end-of-life caregiving (Everyday)	579	(70.5)	285	(49.2)	294	(50.8)	2.94	0.09
Dissatisfaction with end-of-life caregiving								
Knowledge of physical symptoms and management	235	(28.6)	130	(55.3)	105	(44.7)	9.01	<0.01
Professional supports for physical symptoms and management	177	(21.6)	104	(58.8)	73	(41.2)	12.31	<0.01
Knowledge of psychological symptoms and management	228	(27.8)	119	(52.2)	109	(47.8)	3.20	0.08
Professional supports for psychological symptoms and management	208	(25.3)	122	(58.7)	86	(41.3)	14.99	<0.01
Time spent communicating with patients	169	(20.6)	99	(58.6)	70	(41.4)	10.93	<0.01
Dissatisfaction with physicians' end-of-life care								
Treatment of physical symptoms	67	(8.2)	49	(73.1)	18	(26.9)	19.44	<0.01
Treatment of psychological symptoms	119	(14.5)	71	(59.7)	48	(40.3)	8.66	<0.01
Time spent communicating with patients	191	(23.3)	104	(54.5)	87	(45.5)	5.21	<0.01
Time spent communicating with patients' families	232	(28.3)	123	(53.0)	109	(47.0)	4.17	0.05

Fisher's exact test was performed when the sample number was less than 10. All variables were coded as: 0 = absence, 1 = presence.

Table 4. Factors associated with potential psychiatric disorders among bereaved spouses of cancer patients: multivariate logistic regression analysis

Variables	Beta	SE	OR	95% CI	p
Deceased patients' characteristics					
History of usage of psychiatric consultation service	0.42	0.20	1.52	1.02–2.26	0.04
Stomach cancer	0.63	0.30	1.87	1.04–3.38	0.04
Bereaved spouses' characteristics					
Age (< 65 years)	0.72	0.17	2.06	1.47–2.88	<0.01
Time since bereavement (< 3 years)	0.46	0.16	1.58	1.15–2.17	<0.01
Physical illness under treatment	0.82	0.17	2.26	1.62–3.16	<0.01
History of any psychiatric disorder prior to the patient's death	1.16	0.33	3.19	1.68–6.06	<0.01
Dissatisfaction with end-of-life caregiving					
Knowledge of physical symptoms and management	0.32	0.18	1.38	0.97–1.96	0.07
Time spent communicating with patients	0.44	0.20	1.55	1.05–2.30	0.03
Dissatisfaction with physicians' end-of-life care					
Treatment of physical symptoms	1.24	0.31	3.44	1.89–6.26	<0.01

Beta values indicate standardized regression coefficients on the final model after backward elimination. All variables were coded as: 0 = absence, 1 = presence.
SE, standard error; OR, odds ratio; CI, confidence interval.

For the dissatisfaction with EOL care, 'dissatisfaction with physician's treatment of physical symptoms' was the most highly associated with potential psychiatric disorders (OR = 3.44). Unrelieved pain of female cancer patients during their last months of life showed a positive association with psychological morbidity such as sleep disorders in the widowers 4–5 years after bereavement [29]. Additionally, EOL care discussions are associated with less aggressive medical care, such as ventilation and resuscitation and less major depressive disorders in bereaved caregivers [15]. Therefore, satisfactory discussions about physical treatment in EOL care are helpful not only for the patients but also for the caregivers' psychological adjustment. Another factor, 'dissatisfaction with time spent communicating with patients' was significantly associated (OR = 1.55). A recent systematic review of communication with terminally ill patients and their families [30] indicated a lack of quantitative study. Communication skills training for healthcare professionals to improve discussions between patients and caregivers about EOL issues fostering realistic forms of hope is an essential future task for preventive intervention of spousal morbidity after bereavement [30].

We derived several implications for practice and research. In practice, we could obtain the following several indicators for early detection of high-risk spouses prior to the patient's death: 'patients using psychiatric consultation service', 'patients with stomach cancer', 'bereaved with a history of psychiatric disorder', 'dissatisfaction with time spent communicating with patients', and 'dissatisfaction with physician's treatment of physical symptoms'. Along with the early detection of spouses with these risk factors, nurse-assisted [31] or pharmacist-assisted [32] psychiatric referral programs using the 'Distress and Impact Thermometer' might be useful for directly evaluating psychological distress among spouses in EOL practice. In research, we could obtain the following possible strategies for preventive intervention of spousal morbidity after bereavement: assistance for improving 'discussions with physicians about physical treatment in EOL care' and 'discussions between patients and caregivers about EOL issues' would be effective. Development of communication skills training for healthcare professionals to improve these discussions must be considered in future research.

For the study limitations, first, the lack of an exact response rate was a critical methodological limitation. Nevertheless, we believe our estimated sample rate (31%) was adequate because the population of bereaved spouses included those who had died after the patient's death. Second, two sample biases might exist. One was caused by the data collection site, a single cancer center in Japan. However, we do not believe that this institutional bias had a serious effect on the representation of Japanese bereaved spouses of cancer patients because 90% of cancer patients in Japan die in a hospital [19]. In addition, the bereaved with high impaired mental health might have been more motivated to take part in the study. This might have resulted in an inflated number of potential psychiatric disorders. Third, this was a cross-sectional study, and we could not discuss the time course of the prevalence or any causality between impaired mental health and associated factors. In addition, it remains possible that there was a recall bias in answering the question about dissatisfaction with EOL care because it was such a long period for a retrospective report by the bereaved who had lost their partner several years earlier. Fourth, other important factors were not investigated in this study, such as the bereaved spouse's 'style of attachment to the deceased', 'function level among family members', 'perception of the dying process and whether this was traumatic', and 'available social support'. Finally, we have no objective data on EOL care; individuals whose spouses died 7 years ago would likely have had a very different experience in the oncology care setting compared with those whose spouses died more recently.

Conclusions

Nearly half the bereaved spouses showed potential psychiatric disorders even 7 years after bereavement. Patients' psychological distress, bereaved spouses' history of psychiatric disorder, and dissatisfaction with EOL care were indicators of high-risk spouses.

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Conflicts of interest

All authors declare that the answers to the questions on your competing interest form are all 'No' and therefore have nothing to declare.

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ORIGINAL ARTICLE

Strontium-89 (Sr-89) chloride in the treatment of various cancer patients with multiple bone metastases

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Abstract
Background Although the use of Sr-89 chloride in the treatment of patients with prostate and breast cancer has been widely reported, little information is available about its use for other malignancies. Here, we retrospectively analyzed the clinical profile of Sr-89 chloride in various patients with painful bone metastases.
Methods Entry criteria were a pathologically proven malignancy, clinically diagnosed multiple bone metastases, and adequate organ function. Sr-89 chloride (Metastron) was given by single intravenous infusion at 2 MBq/kg over 2 min. Self-reported outcome measures were used as a response index, including pain diary data on a 0–10 numeric rating scale (NRS).
Results Fifty-four consecutive patients with painful bone metastases were treated with Sr-89 chloride at the National Cancer Center Hospital East between March 2009 and July 2011, consisting of 26 with breast/prostate cancer and 28 with other malignancies (lung 8, head and neck 6, colorectal 6, others 8). Thirteen (24 %) patients experienced a transient increase in pain, which was categorized as a flare-up response. Grade 3–4 anemia was observed in 6 patients, 3 of whom required blood transfusion. Regarding efficacy, response rates and complete response rates were 71.2 % and 34.6 %, respectively, and time to response from the initiation of treatment was 36 days (range, 13–217). No significant difference in response rates was seen between patients with breast/prostate cancer and other cancers (breast/prostate 69.2 %, other 73.1 %; $p = 0.76$).
Conclusions As in patients with breast and prostate cancer, Sr-89 chloride is a promising agent for the treatment of painful bone metastases in patients with various other malignancies.

Keywords Palliative care · Radiation oncology · Radiation therapy · Radionuclide · Pain control

Background

The prevalence of painful osseous metastases varies among different types of cancer. Approximately 65 % of patients with prostate or breast cancer and 35 % of those with advanced cancers of the lung, thyroid, and kidney develop symptomatic skeletal metastases. The management of bone pain in these patients remains challenging, and no standardized procedures have yet been adopted. In patients with multifocal osteoblastic metastases, systemic administration of radiopharmaceuticals is the preferred adjunctive therapy for pain palliation.

Similar to calcium, strontium is a divalent cation that is incorporated into hydroxyapatite in bone after intravenous injection [1]. Sr-89 chloride (Metastron) is the first U.S.

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Food and Drug Administration-approved radiopharmaceutical for bone pain palliation. The therapeutic effect derives from the beta particles, which have an energy penetration range of up to 6–7 mm in soft tissues and 3–4 mm in bone [2]. Sr-89 has a half-life of 50.5 days, and decays to stable yttrium-89, emitting high-energy beta particles (E_{max}, 1.46 MeV) and 0.01 % of gamma-rays (910 keV). Administration poses no radiation risk to others, and patients can accordingly be treated on an outpatient basis. Studies of Sr-89 pharmacokinetics have demonstrated that plasma clearance is variable (1.6–11.6 l/day), with overall total-body retention of 20 % in a healthy population at 90 days after injection, particularly in the normal skeleton. Osteoblastic lesions show as much as five times greater radiopharmaceutical uptake and more prolonged retention than areas of normal bone in the same patient (lesion/normal bone ratio, 5:1) [3, 4].

Although the clinical profile of Sr-89 for prostate or breast cancer patients has been widely described [3, 5–9], little information is available concerning patients with other malignant diseases. Here, we conducted a retrospective analysis to clarify the clinical profile of Sr-89 in patients with multiple bone metastases arising from various other cancers.

Patients and methods

Patients

Entry criteria were a pathologically proven malignancy, clinical presence of multiple bone metastases detected by bone scintigraphy, and adequate organ function.

Patients eligible for external-beam radiotherapy (RT) or surgery were basically excluded from Sr-89 candidates.

Written informed consent for treatment was obtained from all patients before the initiation of treatment. This study was approved by the Institutional Review Board of National Cancer Center Hospital, Japan.

Pretreatment evaluation

All patients underwent a complete blood count and serum chemistry testing at entry. Patients who fulfilled any of the following criteria were ineligible: (1) white blood cell count less than 2,000/mm^3; (2) platelet count less than 75,000/mm^3; (3) hemoglobin less than 9 g/dl; and (4) serum creatinine greater than 2.0 mg/dl or creatinine clearance less than 30 ml/min. All patients underwent bone scintigraphy before treatment. Information about pain and analgesic effect was obtained by physician interview in accordance with standard NRS practice.

Protocol treatment

Sr-89 chloride (Metastron) was given by single intravenous infusion at 2 MBq/kg over 2 min followed by a 20-ml saline flush. Premedication was not routinely performed.

Follow-up, response evaluation, and toxicity

Patients visited the outpatient clinic for a complete blood test and interview every 2 weeks from the initiation of treatment until 2 months after treatment. Self-reported outcome measures were used as response index, including pain diary data on a 0–10 numeric rating scale (NRS) [10, 11]. Complete response (CR) was defined as a minimum NRS of 10 % or less than that at the initiation of treatment, partial response (PR) as a minimum of 50 % or less than that at the initiation of treatment, and no response (NR) as a minimum NRS of equal to or greater than that at the initiation of treatment.

Toxicities were graded using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. Biweekly follow-up was continued until toxicities were easily manageable.

Statistical analysis

Survival curves were estimated using the Kaplan–Meier product-limits method with the log-rank test. Overall survival was calculated from the start of treatment to the date of death or last confirmed date of survival. Survival time was censored at the last confirmation date if the patient was alive. Univariate analysis was conducted using the log-rank test.

Results

Patient characteristics

Fifty-four consecutive patients with painful bone metastases were treated with Sr-89 chloride at the National Cancer Center Hospital East between March 2009 and July 2011. All patients were reviewed. Patient characteristics are listed in Table 1. Twenty-six patients (48 %) had breast or prostate cancer. Twenty-six (48 %) had received chemotherapy in the 6 months before the initiation of treatment, among whom the median interval between the last chemotherapy and protocol treatment was 87 days (range, 0–164). Thirty-one patients had received prior palliative radiotherapy for bone metastases.

Of the patients, 23 (43 %) had received bisphosphonate therapy before Sr-89 administration, and all these patients

Table 1 Patient characteristics

	Total	Breast/prostate	Other
Number of patients	54	26	28
Age, median (range) (years)	64 (34–89)		
Gender, male/female	25/29	12/14	13/15
PS, 0–1/2/3	22/23/9	14/9/3	8/14/6
Primary site			
Breast		15	
Prostate		11	
Lung			8
Head and neck			6
Colorectal			6
Other			8

PS performance status

Table 2 Toxicity

	Grade (CTCAE ver. 4.0)		
	3	4	% 3/4
Leucopenia	0	1	1.8
Neutropenia	0	1	1.8
Anemia	5	1	11.1
Thrombocytopenia	2	2	7.4

CTCAE common terminology criteria for adverse events

continued to receive bisphosphonates during and after Sr-89 administration. Sixteen (70 %) of the breast/prostate cancer patients received bisphosphonate therapy, although only 7 patients (25 %) of patients with other cancers received therapy.

Toxicity

Thirteen (24 %) patients experienced a transient increase in pain, which was classified as a flare-up response. Profiling of other nonhematological toxicities was hampered by the frequent use of additional supportive interventions during and after protocol treatment, including morphine or other medications. Hematological toxicity is summarized in Table 2. Grade 3–4 anemia was observed in 6 patients, 3 of whom required blood transfusion within 2 months after protocol treatment. One patient developed disseminated intravascular coagulation (DIC), which might have been related to either Sr-89 administration or primary disease progression or to both.

Efficacy

Two patients were excluded from response evaluation because of sudden death unrelated to the use of Sr-89

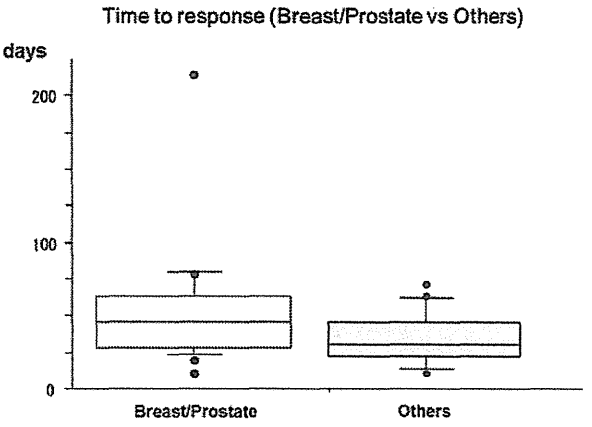

Fig. 1 Time to response (breast/prostate cancer vs. other). Time to response was calculated from the initiation of treatment to the day of pain relief (≥PR). Median time to response in breast/prostate cancer patients was 46 days (range, 13–217); that in other cancer patients was 31 days (range, 14–73). There was no significant difference between breast/prostate cancer patients and others

chloride. One patient with lung cancer died 7 days after Sr-89 administration. Hepatic failure caused by liver metastases was considered to be the main cause of death. Another patient with gastric cancer died 31 days after Sr-89 administration. At first visit after Sr-89, his performance status was 2. However, after the first visit, his condition was rapidly worsened by cachexia.

Overall response rate was 71.2 % and CR rate was 34.6 %. Median time to response was 36 days (range, 13–217 days). Median time to response in breast/prostate cancer patients was 46 days (range, 13–217), whereas that in other cancer patients was 31 days (range, 14–73) (Fig. 1).

Analgesic use at 2 months after treatment was decreased for only 11.5 % of patients. With a median follow-up period of 6.8 months, median survival time was 6.1 months and the 1-year overall survival rate was 28.0 %. Median survival time was significantly longer in patients with breast or prostate cancer than in those with other malignancies (Fig. 2).

Next treatment after Sr-89

After Sr-89 treatment, 9 patients received chemotherapy as a next treatment. The remaining 43 patients received best supportive care. Of those, 12 patients received palliative radiotherapy for bone metastases; median time from Sr-89 to next radiotherapy was 48 days (range, 13–252 days).

Predictive factors

Age, primary site (breast/prostate vs. others), history of chemotherapy, and onset of flare-up response were

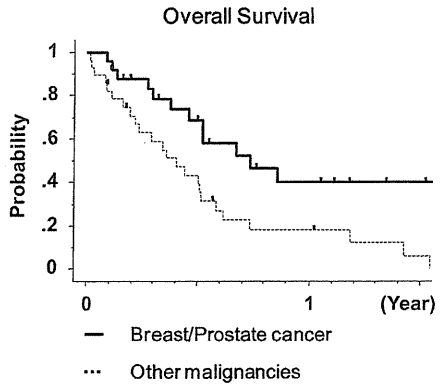

Fig. 2 Overall survival. Median survival time and 1-year survival rate were 8.0 months and 39.9 %, respectively, in patients with breast/prostate cancer, and 4.9 months and 17.9 % in those with other cancers. Overall survival was significantly longer in patients with breast/prostate cancer than in patients with other cancers ($p = 0.008$)

Table 3 Univariate analysis of predictive factors associated with response

	n	RR (%)	*P* value	HR (95 % CI)
Age (years)				1.01 (0.96–10.6)
Gender				
Male	23	73.9	0.70	0.78 (0.23–2.65)
Female	29	69		
Primary site				
Breast/prostate	26	72	0.76	1.21 (0.36–4.02)
Other	26	68.2		
Prior chemotherapy				
Yes	25	53.8	0.90	0.92 (0.28–3.07)
No	27	76.9		
Flare-up response				
Yes	13	53.8	0.12	2.86 (0.76–10.7)
No	39	76.9		

RR reponse rate, *HR* hazard ratio, *CI* confidence interval

investigated in univariate analysis (Table 3), but no significant predictive factor was identified.

Discussion

The clinical profile obtained in this study suggests that Sr-89 chloride may be of benefit in the treatment of painful bone metastases, not only in patients with breast and prostate cancer but also in those with various other malignancies.

Previous studies have identified hematological toxicity as one of the main side effects of Sr-89 chloride [6, 12, 13]. Hematological toxicities were present in the present study also but were of acceptable severity, albeit that the frequency of anemia was slightly higher than in previous reports. Further, the incidence of flare-up response was higher than with radiotherapy. Previous reports have shown similar results [6, 12], which appears to support the hypothesis that the incidence of flare-up response increases with increasing volume of bone metastases.

Among other findings, overall response rate was 71.2 % and CR rate was 34.6 %. Overall response rate was 69.2 % in breast and prostate cancer and 73.1 % in other cancers. These results showed that Sr-89 chloride had definite benefit in patients with painful bone metastases.

Our present results are of particular clinical valuable given the relative paucity of information about the clinical profile of Sr-89 chloride in cancers other than breast and prostate.

In this study, we used self-reported outcome measures as response index, including pain diary data on a 0–10 numeric rating scale (NRS). However, analgesic use was decreased for only 11.5 % of all patients at 2 months after treatment, and considerable discrepancy was seen between the results calculated by the diary and interview response indexes.

In clinical practice, analgesic use is seldom decreased even when the patient reports a decrease in bone pain. There are two major reasons for this: first, analgesics may also be required for other pain; and second, a decrease in analgesic use carries the risk that pain will recur. The recurrence of pain is a fatal outcome, particularly in patients receiving best supportive care. For these reasons, because a change in the amount of analgesics used does not reflect the response to Sr-89 chloride, we consider that this variable should not used as an index in clinical practice.

The predictive factors of response to Sr-89 are still controversial. Some investigators have found a better response in patients with good condition, and a poorer response with far-advanced metastatic disease [9, 14–16]. On the other hand, in some reports no significant difference was seen in patient background between responders and non-responders [17, 18].

In the present study, age, primary site (breast/prostate vs. other), history of chemotherapy, and onset of flare-up response were investigated in univariate analysis, but no significant predictive factor was identified.

The primary site of cancer and treatment history had no impact on the efficacy of Sr-89 chloride in univariate analysis, suggesting that Sr-89 chloride can be considered regardless of the primary site and treatment history.

Two major limitations of this study warrant mention. First, pain-free survival could not be evaluated because of the difficulty in conducting detailed and frequent interviews in patients receiving best supportive care. Second, the small scale and retrospective design of the study meant

that significant predictive factors of efficacy could not be adequately investigated.

Nevertheless, we consider that these results will be valuable for clinicians concerned with the difficult issue of bone pain control, particularly in view of the paucity of other data on this agent.

Conclusion

Sr-89 chloride may useful in the treatment of bone metastasis pain in patients with various malignancies, as it is in those with breast and prostate cancer.

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Conflict of interest We have no conflict of interest.

Ethical standard In the present study, written informed consent for treatment was obtained from all patients before the initiation of treatment. This study was approved in Institutional Review Board of National Cancer Center Hospital, Japan.

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RET-targeting molecular stratified non-small-cell lung cancers

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Abstract: Recent advances in lung cancer genomics have successfully characterized therapeutic targets of lung cancer. *RET* fusion gene products are among the newest target molecules for lung adenocarcinoma. Preclinical findings and preliminary reports regarding potential tumor control by RET-targeting multi-kinase inhibitors encourage further clinical trials. The infrequent prevalence of *RET* fusion gene-positive cases may be a major obstacle hindering the development of RET-targeted therapy. Thus, it is necessary to recruit appropriate participants for trials to develop an efficient *RET* fusion gene detection system to achieve targeted therapy for lung adenocarcinomas stratified by this molecular target.

Keywords: Lung adenocarcinoma; *RET* fusion gene; clinical trial

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Cancer genomics studies involving next generation sequencing (NGS) technology have successfully characterized therapeutic targets of lung cancer. Among lung adenocarcinoma genomes, activating mutations in *EGFR*, *ERBB2*, *KRAS* and *BRAF* as well as gene fusions of *ALK*, the products of which activate the autonomous proliferation of cancer cells via the Ras-MAPK pathway, have been regarded as so-called "driver mutations" (1). It is also known that these alterations exist in a mutually exclusive manner. In addition to these well-characterized driver mutations, independent groups from Japan, Korea and the USA recently found novel chromosome ten inversions that produce fusion genes containing the receptor tyrosine kinase encoding gene *RET* (2-5). Three of four groups applied NGS to determine the fusion genes. Kohno *et al.* and Ju *et al.* used cDNA samples from known driver-negative lung adenocarcinoma specimens for whole transcriptome sequencing to screen novel fusion gene products (2,3). Lipson *et al.* designed a custom target capture genomic DNA sequencing panel targeting the exons and introns of genes encoding previously reported cancer-related kinases and screened lung and colorectal cancer genomes (5).

By using different strategies, these groups identified the same *KIF5B-RET* fusion gene. In these primary reports, the authors found that *RET* fusion gene products are aberrantly expressed in tumor cells. Exogenously overexpressed RET fusion kinases are constitutively active and have transforming activity. Multi-kinase inhibitors, which reportedly inhibit RET, effectively suppress the growth advantage and transforming activity of RET fusion kinases (3-5). Further screening of lung adenocarcinoma-derived cell lines found that LC/2-ad cells, which were established from a pleural effusion from a Japanese patient with lung adenocarcinoma, expressed a *CCDC6-RET* fusion gene (6,7). In addition to experiments with exogenously overexpressed fusion RET, vandetanib, a RET-inhibiting multi-kinase inhibitor, successfully inhibited downstream signals and exhibited significant anti-tumor effects *in vitro* and *in vivo*.

These findings strongly encourage the development of RET-targeted therapy for lung adenocarcinoma. Currently, five independent, open-label, single-arm, phase II studies have begun to assess the therapeutic effects of vandetanib (ZD6474), cabozantinib (XL184), lenvatinib (E7080) and ponatinib (AP24534) (*Table 1*). Drilon *et al.* reported promising results for the first three cases of their clinical trial, which investigated the efficacy of cabozantinib (8). In addition, a case report from Switzerland reported that

Table 1 Ongoing clinical trials of RET-targeting therapies with RET fusion gene-positive NSCLC

Trial ID	Drug (pharmaceutical company)	Study design	Primary end-point	Enrolment (cases)	Study start
NCT01639508	Cabozantinib/XL184 (Exelixis)	Open-label, single arm	Response rate	25	July 2012
UMIN000010095	Vandetanib/ZD6474 (AstraZeneca)	Open-label, single arm	Response rate	17	February 2013
NCT01823068	Vandetanib/ZD6474 (AstraZeneca)	Open-label, single arm	Response rate	17	April 2013
NCT01877083	Lenvatinib/E7080 (Eisai)	Open-label, single arm	Response rate	20	April 2013
NCT01813734	Ponatinib/AP24534 (ARIAD)	Open-label, single arm	Response rate	20	June 2013

vandetanib induced the remission of metastatic *KIF5B-RET* fusion gene-positive lung tumors (9). However, the infrequent prevalence of *RET* fusion gene-positive cases is a major obstacle hindering the further development of RET-targeted therapy. Primary and subsequent studies including a report by Wang *et al.* screened approximately 5,000 lung adenocarcinoma cases in total (10,11). *RET* fusion gene-positive cases were found in 1-2% of all non-small cell lung cancer (NSCLC) patients in Asian and European populations. Based on these estimations, more than 1,000 cases must be screened to identify 10 to 20 *RET* fusion gene-positive cases for proof-of-concept phase II studies. When efficacy is estimated with studies involving a larger number of cases, the number of pre-screening participants is also greater.

The clinicopathological features that characterize *RET* fusion gene-positive cases may help identify patients who should be subjected to further genetic screening. Most of the positive cases are adenocarcinomas, but several cases involve other histological types of NSCLC, such as adenosquamous carcinoma. The *RET* fusion is most likely to occur in young and/or never/light-smoker patients. Lung adenocarcinomas harboring *KIF5B-RET* fusions have well or moderately differentiated histological features similar to those harboring *EGFR* mutations, whereas lung adenocarcinomas harboring *CCDC6-RET* fusions often have signet-ring and mucinous cribriform features similar to *EML4-ALK* fusion-positive lung adenocarcinomas (10,11). These findings suggest a difficulty in distinguishing *RET* fusion gene-positive lung adenocarcinomas from commonly observed lung adenocarcinomas in Asian countries by histopathological diagnosis. Thus, appropriate genetic testing is mandatory for selecting *RET* fusion gene-positive cases.

Investigators have made much effort progress in recruiting adequate numbers of participants for prescreening for the above-mentioned phase II studies. The LURET (Lung Cancer with RET rearrangement) study, led by Dr. Koichi Goto at National Cancer Center Japan (UMIN00001009), evaluates the efficacy of vandetanib in 17 patients with *RET* fusion gene-positive NSCLC. The multi-kinase inhibiting spectrum of vandetanib includes EGFR, and VEGFR and RET. Although the therapeutic efficacy of vandetanib in advanced NSCLC patients was previously evaluated in "all-comer" clinical trials, significantly better therapeutic effects of vandetanib compared to pre-existing therapeutic regimens was not shown. We assume that another clinical trial recruiting only *RET* fusion gene-positive cases is necessary to evaluate the vandetanib effects. To recruit participants, a consortium designated LC-SCRUM (Lung Cancer Genomic Screening Project for Individualized Medicine in Japan) has been established. In LC-SCRUM, frozen biopsy tissues or pleural effusions from patients with non-squamous NSCLC without an *EGFR* mutation are curated from 136 hospitals throughout Japan, and *RET* fusion genes are detected using a combination of RT-PCR and FISH. Multiplex RT-PCR primers are designed to detect all of the previously described *KIF5B-RET* and *CCDC6-RET* variants. The positive cases are then subjected to break-apart and fusion FISH to validate the RT-PCR results. Cases positive by RT-PCR and FISH are eligible for the LURET study.

As Wang *et al.*, mentioned in their report, standard methods for the detection of gene fusions, including RT-PCR, FISH and immunohistochemistry (IHC), have difficulty detecting RET fusion genes and their products (10). RT-PCR exhibits preferable sensitivity and specificity for detecting known fusion gene cDNA, but it is usually insufficient for detecting new partners or isoforms. Anti-RET antibodies that specifically distinguish overexpressed RET fusion proteins have not been generated. Although FISH is currently the most effective diagnostic technology for detecting chromosomal rearrangements, the high cost and need for technical expertise limit its practical application.

We should also take into account the cost efficiency of *RET* fusion gene detection, which only benefits the 1% of

cases with a *RET* fusion gene. The detection of rare fusion genes is not just a pecuniary loss, it also wastes precious tissue samples obtained by biopsy or archived surgically resected specimens. To resolve these difficulties, genomic testing of lung adenocarcinoma driver mutations should evolve from single gene testing to multiplex genetic testing. Several technologies, including digital PCR and NGS-based target-capture sequencing, should be preferable candidates for future *in vitro* diagnostic systems. Although these technologies are still immature in their robustness and cost efficiency, these next-generation technologies must be positively applied to clinical diagnosis and may help in establishing a basis for the development of targeted therapy for lung cancer treatment.

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Effect of a poly(ADP-ribose) polymerase-1 inhibitor against esophageal squamous cell carcinoma cell lines

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Key words

DNA repair, esophageal cancer, poly(ADP-ribose) polymerase inhibitor, RNF8, γ-H2AX

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Effective molecular target drugs that improve therapeutic efficacy with fewer adverse effects for esophageal cancer are highly anticipated. Poly(ADP-ribose) polymerase (PARP) inhibitors have been proposed as low-toxicity agents to treat double strand break (DSB)-repair defective tumors. Several findings imply the potential relevance of DSB repair defects in the tumorigenesis of esophageal squamous cell carcinoma (ESCC). We evaluated the effect of a PARP Inhibitor (AZD2281) on the TE-series ESCC cell lines. Of these eight cell lines, the clonogenic survival of one (TE-6) was reduced by AZD2281 to the level of DSB repair-defective Capan-1 and HCC1937 cells. AZD2281-induced DNA damage was implied by increases in γ-H2AX and cell cycle arrest at G2/M phase. The impairment of DSB repair in TE-6 cells was suggested by a sustained increase in γ-H2AX levels and the tail moment calculated from a neutral comet assay after X-ray irradiation. Because the formation of nuclear DSB repair protein foci was impaired in TE-6 cells, whole-exome sequencing of these cells was performed to explore the gene mutations that might be responsible. A novel mutation in RNF8, an E3 ligase targeting γ-H2AX was identified. Consistent with this, polyubiquitination of γ-H2AX after irradiation was impaired in TE-6 cells. Thus, AZD2281 induced growth retardation of the DSB repair-impaired TE-6 cells. Interestingly, a strong correlation between basal expression levels of γ-H2AX and sensitivity to AZD2281was observed in the TE-series cells (R^2 = 0.5345). Because the assessment of basal DSB status could serve as a biomarker for selecting PARP inhibitor-tractable tumors, further investigation is warranted.

Esophageal carcinoma is the sixth most common cause of cancer-related deaths worldwide and is associated with a poor prognosis.[1] Surgical therapies of resectable esophageal cancer exhibit a 5-year survival rate ranging from 20% to 27%.[2–4] Less invasive therapies that preserve the esophagus have also been introduced. Endoscopic therapies, such as endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), have been adopted for early esophageal cancer and have achieved favorable outcomes, but postoperative esophageal stricture frequently occurs after these treatments. Furthermore, intensive follow-up is necessary to manage new heterochronous lesions.[5–7] Chemoradiotherapy (CRT), which combines radiation, 5-fluorouracil (5-FU) and cisplatin (CDDP), is a promising therapeutic alternative to esophagectomy with a survival rate equivalent to that of surgical therapies.[8,9] However, the acute and late adverse effects of chemoradiotherapy, including pancytopenia and pneumonitis, still require consideration. There is a demand for effective molecular target drugs for esophageal cancer that combine an improved therapeutic efficacy with fewer adverse effects.

Poly(ADP-ribose) polymerase (PARP) inhibitors induce the accumulation of DNA single-strand breaks (SSB), which cause the formation of DNA double-strand breaks (DSB) after the stalling and collapse of progressing DNA replication folks.[10] Though DSBs are repaired by the error-free homologous recombination repair (HRR) pathway in non-tumor cells, they remain unrepaired and induce lethality in HRR-defective tumor cells.[11,12] Based on this mechanism, PARP inhibitors have been proposed as low toxicity agents for HRR-defective tumors. BRCA1 and BRCA2 are key components of the HRR machinery, and the abnormality of these genes is known to cause sporadic and hereditary breast and ovarian cancers.[13] Consistent with this, PARP inhibitors have been developed for breast and ovarian cancers. In addition, an increasing number of biomarker candidates that predict the sensitivity of a tumor to PARP inhibitors have been reported.[14–18]

Esophageal carcinoma is histologically classified into squamous cell carcinoma (ESCC) and adenocarcinoma; the former is common in East Asia. Although the direct relevance has not been well investigated, several findings suggest that a defect in the HRR pathway contributes to the tumorigenesis of ESCC. The risk of esophageal and head and neck squamous carcinoma is increased among Fanconi anemia (FA)

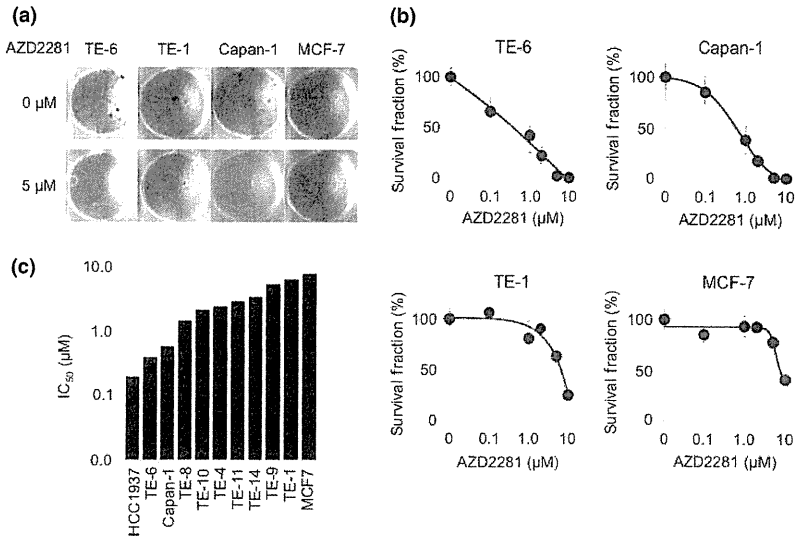

Fig. 1. Sensitivity of TE-series cell lines to a poly (ADP-ribose) polymerase (PARP) inhibitor, AZD2281. (a) TE-1, TE-6, Capan-1 (BRCA2-deficient) and MCF7 (wild-type BRCA) cells were treated with or without AZD2281 at the indicated concentrations for at least seven doubling times. Cells were fixed and stained with crystal violet and the number of colonies was counted. (b) Sensitivity to AZD2281 was evaluated by clonogenic assay. Colonies consisting of more than 64 cells were counted and the survival fraction was estimated. Three independent experiments were carried out. The data represent the average and standard deviations. (c) IC_{50} (μM) value of AZD2281 in eight TE series, MCF7, HCC1937 and Capan-1 cells measured by the clonogenic assays.

patients whose HRR pathway was disturbed due to FA-predisposing gene alterations.[19,20] In addition, recently reported whole-exome sequencing data from 74 head and neck SCCs revealed that more than half of SCC cases harbored mutations in genes involved in DNA repair.[21] Therefore, we assume that some fraction of ESCCs harbor DSB repair defects and might be favorable targets of PARP inhibitors. The aim of this study was to examine the efficacy of a potent PARP-1 inhibitor in a series of ESCC cell lines established from Japanese patients.

Materials and Methods

Complete materials and methods were described in the supplementary information (Data S1).

Purchased materials. A PARP inhibitor, AZD2281 (Olaparib) and BSI-201 (Iniparib) were purchased from Selleck Chemicals (Houston, TX, USA). The TE-1, TE-4, TE-6, TE-8, TE-9, TE-10, TE-11 and TE-14 cell lines were purchased from the Riken BioResource Center (Tsukuba, Japan). The Capan-1, HCC1937, MDA-MB-436 and MCF-7 cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).

Clonogenic assays. A total of 500–2000 cells were cultured with AZD2281- or vehicle-containing media. After 10–16 days, cells were fixed and stained with crystal violet. Colonies consisting of more than 64 cells were subsequently counted.

Immunoblotting analysis. The treated cell lysates were separated by 15% SDS-PAGE and the blot was hybridized with the

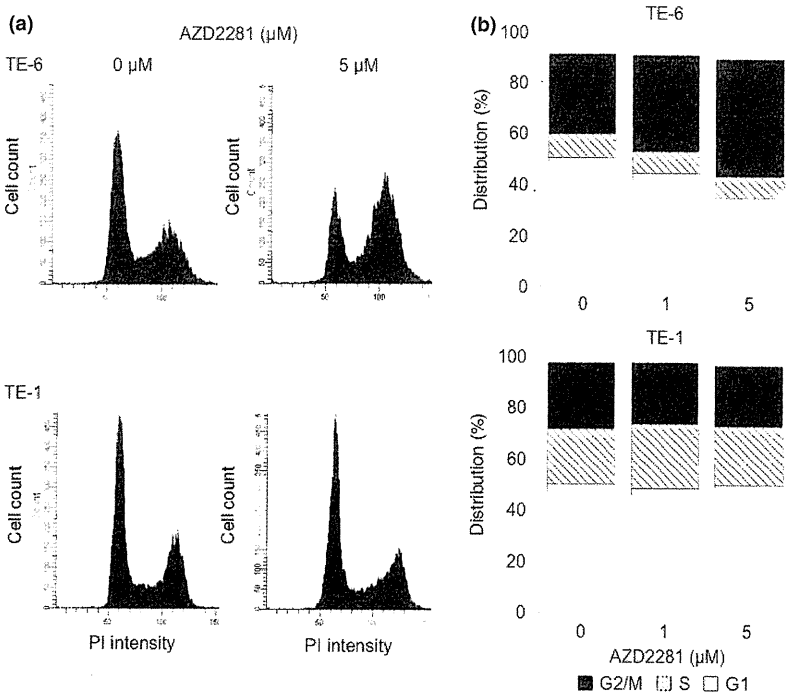

Fig. 2. AZD2281-induced G2/M arrest in TE-6 cells. (a) TE-6 and TE-1 cells were cultured with or without AZD2281 for 24 h. DNA ploidy was assessed by propidium iodide (PI) staining and flow cytometry. (b) The proportion of estimated cell-cycle phases in TE-6 and TE-1 cells treated with or without AZD2281. The data represent the average of three independent experiments.

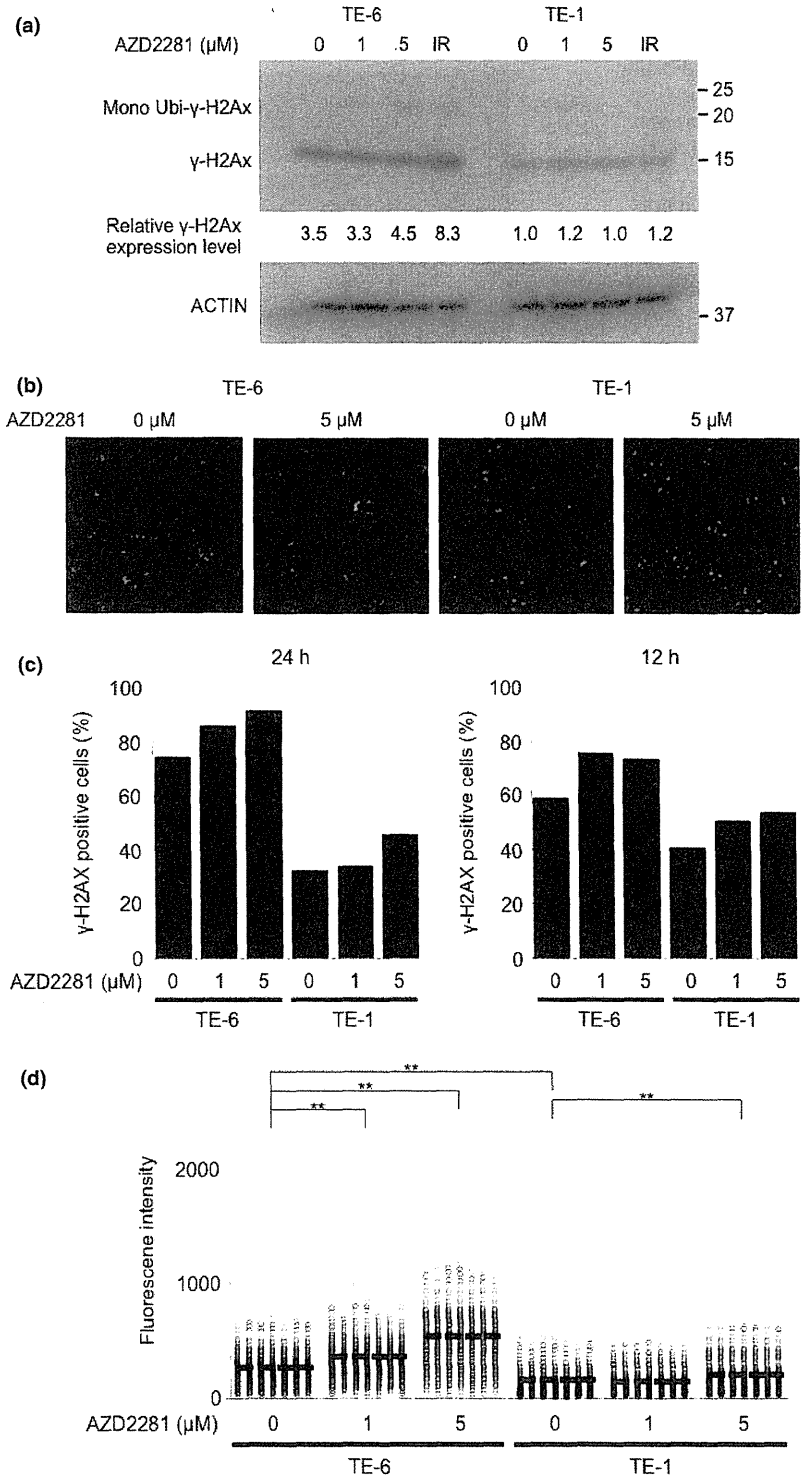

Fig. 3. Increase in double strand breaks (DSBs) in TE-6 cells treated with AZD2281. (a) TE-6 and TE-1 cells were treated with AZD2281 for 24 h and with 5 Gy X-ray irradiation, and γ-H2AX was assessed using Western blotting. The anti-γ-H2AX antibody detected both unubiquitinated (15 kD) and mono-ubiquitinated (23.6 kD) γ-H2AX. (b) TE-6 and TE-1 cells were treated with AZD2281 for 24 h and γ-H2AX was assessed by immunofluorescence. DAPI (blue) and γ-H2AX (red) images were superimposed. (c) Number of the γ-H2AX-positive TE-6 and TE-1 cells treated with or without AZD2281 at the indicated concentrations for 24 h. (d) Scatter diagrams show the fluorescence intensity of individual TE-6 and TE-1 cells treated with or without AZD2281 at the indicated concentrations for 24 h. The lines shown indicated the averages of the data plotted. The data were obtained from at least 500 cells for each condition. $^{**}P < 0.01$ (Student's *t*-test).

phospho-Histone H2A.X (Ser139) (20E3) rabbit monoclonal antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA) and then with a HRP-conjugated secondary antibody (1:50000; Santa Cruz Biotechnology, Dallas, TX, USA). Densitometric analysis was performed with Image-J software (http://imagej.nih.gov/ij/).

Immunofluorescence analysis. To evaluate the formation of DSBs, cells grown on 96-well plates were treated with an anti-γ-H2AX rabbit monoclonal antibody (Cell Signaling Technology) followed by a goat anti-Mouse IgG (H + L) DyLight 549-conjugated secondary antibody (Thermo Scientific, Waltham, MA, USA). γ-H2AX was observed under an ArrayScan HCS System (Thermo Scientific). To evaluate the formation of 53BP1 and RAD51 nuclear foci, cells grown on μ-Dish$^{35\ mm,\ low}$ (ibidi) were treated with an anti-53BP1 rabbit polyclonal antibody (Abcam, Cambridge, UK) or an anti-RAD51 rabbit