Memorial Sloan-Kettering Cancer Center (MSKCC) score [8], which include ECOG PS >1 ($P = 0.001$), low Hb levels ($P = 0.002$), high corrected calcium levels ($P = 0.008$) and high LDH levels ($P < 0.001$), and ≤12 months between diagnosis and initial systemic therapy ($P = 0.037$), were associated with worse OS (Table 2). Multivariate analysis by a Cox proportional hazard model showed that low Hb and high LDH were independently associated with poorer OS among MSKCC scores (Table 3). In addition, brain metastasis and no history of nephrectomy were also associated with poorer OS.

Because the patients included in the present study represent a mixture of treatment naïve and refractory RCC patients, we analyzed these two groups separately. The application of the MSKCC model (using PS, Hb, calcium, LDH and time from diagnosis to initiation of systemic therapy) to stratify patients into three risk groups for the treatment naïve patients (favourable: no risk factors, 12%, $n = 4$; intermediate: one or two risk factors, 59%, $n = 20$; poor: three to five risk factors, 29%, $n = 10$) distinctly separated the OS curves (Fig. 1A). On the other hand, the application of the MSKCC model for the treatment refractory patients [9] (using Hb, calcium and PS) to stratify patients into three risk groups (favourable: no risk factors, 13%, $n = 12$; intermediate: one risk factor, 62%, $n = 13$; poor: two or three risk factors, 25%, $n = 4$) also distinctly separated the OS curves (Fig. 1B).

TABLE 3 Multivariate analyses associated with poor survival

Parameter	Hazard ratio	95% CI	*P*
Laboratory data			
Haemoglobin <LLN	2.658	1.064–7.547	0.044
Lactate dehydrogenase >1.5 × ULN	2.678	1.105–6.490	0.029
Clinical data			
Brain metastasis	6.499	2.277–18.555	0.001
No history of nephrectomy	3.086	1.287–7.407	0.012

LLN, lower limit of normal range; ULN, upper limit of normal range.

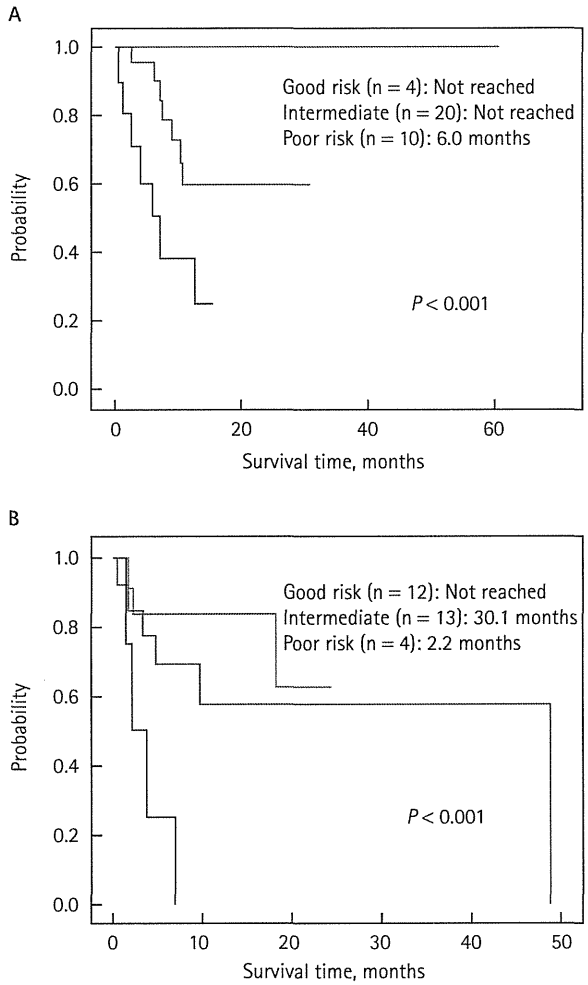

FIG. 1.
Stratification of the overall survival for the patients with metastatic renal cell cancer by the respective Memorial Sloan-Kettering Cancer Center (MSKCC) risk scores. Stratification of the treatment naïve (A) and the treatment refractory (B) patients by MSKCC risk scores.

ADVERSE EFFECTS

All 63 patients experienced treatment-related adverse events, most of which were grade 1 or 2 in severity. The most common grade 3 or 4 adverse events were fatigue (53%), thrombocytopaenia (48%), hand-foot syndrome (16%), anaemia (20%), hypertension (10%) and leucopaenia (9%). Most of these adverse events were manageable and reversible. Although most patients were able to resume therapy after treatment modification, only two (3%) patients and one (1%) patient discontinued because of adverse fatigue and hand-foot syndrome events, respectively. In particular, the incidence of hypothyroidism (76%) was remarkable. Among these patients, levothyroxine had to be administered to maintain thyroid function in 37 (59%) patients.

DISCUSSION

In the present study, we retrospectively analyzed the ORR, PFS, OS and prognostic factors in 63 native Japanese patients, suggesting that the results of the present study could reflect current clinical practice in metastatic RCC in our country. In this cohort, the ORR and clinical benefit (partial response plus stable disease >12 weeks) from sunitinib were 30% and 81%, respectively. These are somewhat lower than the results of a Japanese phase II study (52% and 78%) [5] but slightly better than the results from an expanded-access programme in Western countries (16% and 76%) [10]. The estimated median PFS and OS in the present study were 9.3 and 32.2

© 2011 THE AUTHORS
BJU INTERNATIONAL © 2011 BJU INTERNATIONAL

months, respectively. These are also somewhat lower than the results of the Japanese phase II study (12.2 and 33.1 months for treatment-naïve patients and 10.6 and 32.5 months for cytokine-refractory patients) but slightly better than the results from the expanded-access programme in Western countries (10.9 and 18.4 months) [5,10,11]. In addition, it should be noted that 42% of the patients in the present study were still on treatment, which may have resulted in an underestimation of the ORR, as suggested by a recent analysis showing a higher ORR after longer follow-up [3,12].

It is remarkable that the median OS from the initial systemic therapy of the pretreated patients was 79.6 months. In a phase III randomized clinical trial, sunitinib showed longer PFS and OS compared to interferon-α as a first-line therapy for patients with metastatic RCC [3,4]. Interestingly, the OS was not significantly different between the treatment naïve patients and pretreated patients in the present study (not reached and 24.2 months), in the expanded-access programme (18.4 and 18.1 months) [10] and in the Japanese phase II study (33.1 and 32.5 months, respectively [11]. These results suggest that sunitinib can give a favourable impact as a second-line therapy for patients refractory to cytokine therapy rather than for treatment-naïve patients.

All of the risk factors (ECOG PS >1, low Hb level, high corrected calcium level, high LDH level and ≤12 months between diagnosis and initial systemic therapy) were associated with worse OS in the MSKCC scores, which have been previously identified in patients treated with cytokines (Fig. 1A and Table 2) [8,9]. The application of the respect MSKCC models for the treatment naïve and refractory patients also distinctly separated the OS curves (Fig. 1). This is consistent with recent studies identifying patients who probably will benefit from tyrosine kinase inhibitors [12–15]. These results, as well as those obtained in the present study, indicate that MSKCC scores are associated with the behaviour of the disease rather than with specific forms of therapy. Therefore, MSKCC prognostic factors are still valid for predicting survival in metastatic RCC in the molecular targeted therapy era. However, the distribution of patients according to the MSKCC model is uneven: in the series from the present study, 13%, 62% and 25% of patients belonged to favourable, intermediate and poor risk groups, respectively. The disproportionately large number of patients in the intermediate group suggests that the outcome of this group may be somewhat heterogeneous. The prognostic significance of these factors remains to be verified in a larger study because the present study is only preliminary and has a small number of patients.

Apart from the MSKCC score, brain metastasis and no history of nephrectomy were also independently associated with poorer OS. The cumulative incidence of brain metastases is ≈10%, and these patients are considered to have a poor prognosis (median overall survival, 3–6 months) [16,17]. Although some studies have reported that sunitinib has activity against brain metastasis [18,19], and even against multiple brain lesions [19], there is insufficient information available about the activity of sunitinib for brain metastasis because most clinical trials have excluded patients with brain metastasis. Intracerebral haemorrhage in RCC patients with brain metastases should be considered as a cautious adverse effect to be treated with tyrosine kinase inhibitors, although the incidence of this remains to be reported. We consider radiotherapy as a primary treatment for patients with brain metastasis; thereafter, targeted therapies could be considered. However, in the present study, no patients suffered intracerebral haemorrhage.

Among the 49 patients who underwent nephrectomy, 22 patients did so as a cytoreductive strategy. Upfront cytoreductive nephrectomy, followed by systemic therapy, has been established as the standard care for metastatic RCC in the cytokine era [20,21]. Targeted agents, including sunitinib, have shown improved outcomes compared to cytokine therapy, transforming the treatment strategy of metastatic RCC. Although many studies focus on the role of cytoreductive nephrectomy in combination with targeted agents for patients with metastatic RCC, cytoreductive nephrectomy is still recommended at least for those patients with currently good PS.

All patients experienced treatment-related adverse events, most of which were grade 1 or 2 in severity, and most patients were able to resume therapy after treatment modification. In particular, the incidence of grade 3/4 haematological toxicities, including anaemia (20%), leucopaenia (9%) and thrombocytopaenia (48%), appears to be higher in the present study compared to the previous worldwide phase III clinical trial data [3,4], and also is consistent with the report from the Japanese phase II clinical trial [5,11].

In conclusion, sunitinib has a favourable efficacy/safety profile for Japanese metastatic RCC patients in clinical practice. The estimated median OS was >2 years with acceptable tolerability. In addition, it should be noted that the median OS from the initial systemic therapy of pretreated patients was >6 years. MSKCC prognostic factors appear to be still valid for predicting survival in metastatic RCC in the era of molecular targeted therapy.

ACKNOWLEDGEMENTS

This work was partly supported by the Takeda Science Foundation, the Kobayashi Institute for Innovative Cancer Chemotherapy, and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

CONFLICT OF INTEREST

None declared.

REFERENCES

1 **Hutson TE, Figlin RA.** Novel therapeutics for metastatic renal cell carcinoma. *Cancer* 2009; **115**: 2361–7
2 **Christensen JG.** A preclinical review of sunitinib, a multitargeted receptor tyrosine kinase inhibitor with anti-angiogenic and antitumour activities. *Ann Oncol* 2007; **18**: x3–10
3 **Motzer RJ, Hutson TE, Tomczak P *et al.*** Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**: 115–24
4 **Motzer RJ, Hutson TE, Tomczak P *et al.*** Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 3584–90

© 2011 THE AUTHORS
BJU INTERNATIONAL © 2011 BJU INTERNATIONAL

5 **Uemura H, Shinohara N, Yuasa T *et al.*** A phase II study of sunitinib in Japanese patients with metastatic renal cell carcinoma: insights into the treatment, efficacy and safety. *Jpn J Clin Oncol* 2010; **40**: 194–202

6 **Therasse P, Arbuck SG, Eisenhauer EA *et al.*** New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, national cancer institute of the United States, national cancer institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–1

7 **Payne RB, Little AJ, Williams RB, Milner JR.** Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 1973; **4**: 643–6

8 **Motzer RJ, Murphy BA, Bacik J, Russa P, Mazudmar M.** Interferon-alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol* 2001; **20**: 289–96

9 **Motzer RJ, Bacik J, Schwartz LH *et al.*** Prognostic factors for survival in previously treated patients with metastatic renal cell carcinoma. *J Clin Oncol* 2004; **22**: 454–63

10 **Gore ME, Szczylik C, Porta C *et al.*** Safety and efficacy of sunitinib for metastatic renal-cell carcinoma: an expanded-access trial. *Lancet Oncol* 2009; **10**: 757–63

11 **Tomita Y, Shinohara N, Yuasa T *et al.*** Overall survival and updated results from a phase II study of sunitinib in Japanese patients with metastatic renal cell carcinoma. *Jpn J Clin Oncol* 2010; **40**: 1166–72

12 **Bamias A, Karadimou A, Lampaki S *et al.*** Prognostic stratification of patients with advanced renal cell carcinoma treated with sunitinib: comparison with the Memorial Sloan-Kettering prognostic factors model. *BMC Cancer* 2010; **10**: 45

13 **Choueiri TK, Garcia JA, Elson P *et al.*** Clinical factors associated with outcome in patients with metastatic clear-cell renal cell carcinoma treated with vascular endothelial growth factor-targeted therapy. *Cancer* 2007; **110**: 543–50

14 **Patil S, Figlin RA, Hutson TE *et al.*** Prognostic factors for progression-free and overall survival with sunitinib targeted therapy and with cytokine as first-line therapy in patients with metastatic renal cell carcinoma. *Ann Oncol* 2011; **22**: 295–300

15 **Heng DY, Xie W, Regan MM *et al.*** Prognostic factors for overall survival in patients with metastatic renal cell carcinoma treated with vascular endothelial growth factor-targeted agents: results from a large, multicenter study. *J Clin Oncol* 2009; **27**: 5794–9

16 **Schouten LJ, Rutten J, Huveneers HA, Twijnstra A.** Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma. *Cancer* 2002; **94**: 2698–705

17 **Barnholtz-Sloan JS, Sloan AE, Davis FG, Vigneau FD, Lai P, Sawaya RE.** Incidence proportions of brain metastases in patients diagnosed (1973 to 2001) in the Metropolitan Detroit Cancer Surveillance System. *J Clin Oncol* 2004; **22**: 2865–72

18 **Medioni J, Cojocarasu O, Belcaceres JL, Halimi P, Oudard S.** Complete cerebral response with sunitinib for metastatic renal cell carcinoma. *Ann Oncol* 2007; **18**: 1282–3

19 **Zeng H, Li X, Yao J *et al.*** Multifocal brain metastases in clear cell renal cell carcinoma with complete response to sunitinib. *Urol Int* 2009; **83**: 482–5

20 **Flanigan RC, Salmon SE, Blumenstein BA *et al.*** Nephrectomy followed by interferon alfa-2b compared with interferon alfa-2b alone for metastatic renal-cell cancer. *N Engl J Med* 2001; **345**: 1655–9

21 **Mickisch GH, Garin A, van Poppel H, de Prijck L, Sylvester R.** European Organisation for Research and Treatment of Cancer (EORTC) Genitourinary Group. Radical nephrectomy plus interferon-alfa-based immunotherapy compared with interferon alfa alone in metastatic renal-cell carcinoma: a randomised trial. *Lancet* 2001; **358**: 966–70

Correspondence: Takeshi Yuasa, Department of Urology and Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31, Ariake, Tokyo 135-8550, Japan.
e-mail: takeshi.yuasa@jfcr.or.jp

Abbreviations: **ECOG**, Eastern Cooperative Oncology Group; **Hb**, haemoglobin; **LDH**, lactate dehydrogenase; **MSKCC**, Memorial Sloan-Kettering Cancer Center; **ORR**, objective response rate; **OS**, overall survival; **PFS**, progression-free survival; **PS**, performance status.

© 2011 THE AUTHORS
BJU INTERNATIONAL © 2011 BJU INTERNATIONAL

Leukemia & Lymphoma, May 2012; 53(5): 849–854
© 2012 Informa UK, Ltd.
ISSN: 1042-8194 print / 1029-2403 online
DOI: 10.3109/10428194.2011.635858

informa
healthcare

ORIGINAL ARTICLE: CLINICAL

High thymidine kinase activity is a strong predictive factor for poor prognosis in peripheral T-cell lymphoma treated with cyclophosphamide, adriamycin, vincristine and prednisone

Kazuhito Suzuki, Yasuhito Terui, Kenji Nakano, Eriko Nara, Kentaro Nasu, Kyoko Ueda, Noriko Nishimura, Yuko Mishima, Sakura Sakajiri, Masahiro Yokoyama, Shunji Takahashi & Kiyohiko Hatake

Department of Medical Hematology/Oncology, Cancer Institute Hospital, Tokyo, Japan

Abstract

The prognosis of patients with peripheral T-cell lymphoma (PTCL) treated with cyclophosphamide, adriamycin, vincristine and prednisone (CHOP) is poor, but their laboratory prognostic parameters had not previously been evaluated. We retrospectively reviewed 55 patients with newly diagnosed PTCL treated with CHOP from August 1999 to May 2009 at our institution. We analyzed six laboratory parameters, including thymidine kinase (TK) activity, to evaluate overall survival, which was the primary end-point. In multivariate analysis, the overall survival was significantly worse in patients with high TK activity (hazard ratio 34.8, 95% confidence interval [CI] 1.03–1176.23). The overall response rate among patients with high TK activity was 21.4%, significantly poorer compared with other parameters ($p = 0.001$). High TK activity predicts poor overall survival among patients with newly diagnosed PTCL treated with CHOP. Response to CHOP treatment is significantly decreased in patients with PTCL with high TK activity.

Keywords: Lymphoma and Hodgkin disease, chemotherapeutic approaches, prognostication

Introduction

Chemotherapy for peripheral T-cell lymphoma (PTCL) has never been established, although we commonly use CHOP (cyclophosphamide, adriamycin, vincristine and prednisone), similar to the chemotherapy used for aggressive non-Hodgkin lymphomas such as diffuse large B-cell lymphoma (DLBCL). The 5-year overall survival rate is about 30% when CHOP is administered for the treatment of PTCL [1].

The most valuable prognostic factor for PTCL is the histology. Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS), angioimmunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL) are major subtypes, representing more than 40% of PTCLs in Asia. The 5-year overall survival rates for ALK-positive ALCL, ALK-negative ALCL, PTCL-NOS and AITL are 70%, 49%, 32% and 32%, respectively [1]. Thus, the outcome of PTCL (excluding ALK-positive ALCL) is much poorer than that of DLBCL [1]. In previous studies, several laboratory parameters have been reported as prognostic factors for PTCL [2–10]. PTCLs are a heterogeneous group of neoplasms that behave aggressively and lead to a poor outcome. For patients with PCTL, it is necessary to use prognostic factors to select the best treatment.

The purpose of this study was to investigate laboratory prognostic factors, including serum thymidine kinase (TK) activity, serum soluble interleukin-2 receptor (sIL-2R) level, serum β_2-microglobulin (β2M) level, serum lactate dehydrogenase (LDH) level, serum C-reactive protein (CRP) level and MIB-1 index, in order to evaluate overall survival among patients with PTCL-NOS, AITL and ALCL treated with CHOP. All patients were enrolled in a retrospective single-center study at the Cancer Institute Hospital, Japanese Foundation for Cancer Research.

Materials and methods

Patients

We retrospectively reviewed patients with PTCL newly diagnosed at our institution between August 1999 and May 2009. Subtypes PTCL-NOS, AITL and ALCL were included. All cases of ACLC expressed CD30; cutaneous ALCL was not included. All pathological specimens were reviewed by one hematopathologist. This study was approved by the Independent Ethics Committee/Institutional Review Board at our institution. Patients provided written informed consent before entering the study, which was performed in accordance with the Declaration of Helsinki.

Treatment and assessment of response

All patients received CHOP (cyclophosphamide, 750 mg/m^2, day 1; vincristine, 1.4 mg/m^2, day 1; doxorubicin, 50 mg/m^2, day 1; prednisone, 60 mg/m^2, days 1–5) as induction therapy. We typically administered six cycles of CHOP for PTCL. Three patients received radiotherapy after CHOP. We performed up-front autologous stem cell transplant (ASCT) for patients

Correspondence: Dr. Kiyohiko Hatake, Cancer Institute Hospital, Medical Hematology/Oncology, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan. E-mail: khatake@jfcr.or.jp

Received 8 July 2011; revised 19 October 2011; accepted 21 October 2011

RIGHTSLINK

with the following parameters: international prognostic index (IPI) 3 or more, prognostic index for PTCL-unspecified (PIT) 2 or more; cytotoxic phenotype positive and CD56 positive [2,3].

Evaluations were carried out using the following criteria. Complete remission was defined as the disappearance of all clinical evidence of lymphoma, with no persisting disease-related symptoms. Partial response was defined as a decrease greater than 50% in the sum of the products of the two longest diameters of all measurable lesions, and non-measurable lesions had to decrease by at least 50%. Progressive disease was defined as any increase greater than 25% in the sum of the diameters of any measurable lesions or the appearance of new lesions. Stable disease was considered to be any condition intermediate between partial response and progressive disease.

Prognostic factors

The following parameters were recorded and evaluated for prognosis: serum TK activity, serum sIL-2R level, serum β2M level, serum LDH level, serum CRP level, serum hemoglobin (Hb) concentration and MIB-1 index, as determined using an immunohistochemical technique in archival paraffin-embedded sections. All serum assays were performed on fresh samples before CHOP therapy. The TK assay was performed using a conventional radioenzyme assay at Special Reference Laboratories, Inc. (Japan) [4]. MIB-1 scoring was assessed according to the rate of tumor cells immunohistochemically positive for MIB-1 by semi-quantitative analysis [5,6]. The upper normal limits of serum TK activity and sIL-2R level are 5 IU/L and 519 IU/mL, respectively. The lower normal limit of serum Hb is 12.5 g/dL among males and 11.0 g/dL among females, respectively. We also analyzed other, well-established prognostic factors: age over 60 years, stage of disease more than II, performance status more than 1 and histologic subtype of disease (PTCL-NOS vs. AITL vs. ALCL). Performance status was determined according to the Eastern Cooperative Oncology Group (ECOG) score.

Statistical analysis

The primary end-point was the evaluation of laboratory parameters that predicted poor overall survival among patients with PTCL treated with CHOP. First, univariate analysis was performed for each of the parameters indicated above in order to classify patients with high-risk PTCL. Actuarial survival analysis was performed using the method described by Kaplan and Meier, and curves were compared by log-rank test. All prognostic variables were considered for multivariate analysis for survival. The latter was performed by Cox regression analysis. The poor prognostic significance of laboratory parameters was assessed relative to the well-established prognostic factors by Cox regression. Second, correlations between the overall response rate (partial response plus complete remission rate) with CHOP and laboratory parameters were assessed using the χ^2 test and Fisher's exact test. Finally, we analyzed the correlation between TK activity and other parameters using Pearson's product moment correlation coefficient in order to determine whether high TK activity predicted poor outcome independently. All p-values reported are two-sided, and statistical significance was defined as $p < 0.05$. Statistical analyses were computed using SPSS statistical software (SPSS, Inc., Chicago, IL).

Results

Patients and treatment

Fifty-five patients with newly diagnosed PTCL were entered into this study. The main characteristics are shown in Table I. The median age of the study patients was 57.3 years (range, 16.5–81.8). Diseases were 30 PTCL-NOS (54.5%), 13 AITL (23.6%) and 12 ALCL (21.8%). Median TK activity was 23 IU/L (range, 8–340 IU/L), median sIL-2R level was 2065 IU/mL (range, 446–54 400 IU/mL), median β2M level was 2.36 mg/dL (range, 0.99–10.21 mg/dL), median LDH level was 226 IU/L (range, 136–1018 IU/L) and median CRP level was 0.7 IU/L (range, 0.1–12.3 IU/L). The percentages of patients with MIB-1 index 30% or less and over 30% were 50% each. The median number of cycles of CHOP was six (range, 1–9). Three patients received radiotherapy after three cycles of CHOP. Two of these patients, diagnosed as having PTCL-NOS and AILT with limited disease, received radiotherapy after three cycles of CHOP. The other patient, diagnosed as having ALCL, received radiotherapy for residual disease after six cycles of CHOP. Nine patients received ASCT as salvage therapy. Four patients received up-front ASCT.

Response and survival

Among the 55 patients, 19 patients achieved a complete remission, 15 patients achieved a partial response and 12

Table I. Patient characteristics.

	Patients ($n = 55$)	
	n	%
Age (years)	57.3 (16.5–81.8)	
Gender		
Male	37	67.30
Female	18	32.70
Histology		
PTCL-NOS	30	54.50
AITL	13	23.60
ALCL	12	21.80
Performance status		
0, 1	44	89.80
2, 3, 4	5	10.20
Ann Arbor clinical stage		
I/II	17	31.50
III/IV	37	68.50
IPI		
0, 1, 2	33	73.30
3, 4	12	26.70
PIT		
0, 1	35	76.10
2, 3, 4	11	23.90
TK activity (IU/L)	23 (8–340)	
sIL-2R (IU/mL)	2065 (446–54 400)	
β2M (mg/dL)	2.36 (0.99–10.21)	
LDH (IU/L)	226 (136–1018)	
CRP (IU/L)	0.7 (0.1–12.3)	
MIB-1 index (%)		
0–30	12	50.00
31–90	12	50.00

PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; AITL, angioimmunoblastic T-cell lymphoma; ALCL, ALK-negative anaplastic large cell lymphoma; IPI, international prognostic index; PIT, prognostic index for PTCL-unspecified; TK, thymidine kinase; sIL-2R, soluble interleukin-2 receptor; β2M, β_2-microglobulin; LDH, lactate dehydrogenase; CRP, C-reactive protein.

RIGHTSLINK

had progressive disease. The complete remission rate and the overall response rate were 34.5% and 61.8%, respectively. Five patients could not continue CHOP because of adverse events, including severe pneumonia, herpes zoster and hyperglycemia. The median follow-up time for all patients was 24.3 months. Median overall survival and progression-free survival were 49.7 months (95% confidence interval [CI] 17.7–81.7 months) and 9.1 months (95% CI 1.8–16.4 months), respectively.

Prognostic factors

The analysis of prognostic factors influencing overall survival is shown in Table II. The overall survival was significantly worse in patients with the following prognostic factors: TK activity over 25 IU/L ($p = 0.048$), serum LDH level above the upper normal limit ($p = 0.018$), serum CRP level above the upper normal limit ($p = 0.013$) and MIB-1 index over 30% ($p = 0.011$) according to univariate analysis. Serum sIL-2R level over 3000 U/mL tended to predict poor survival ($p = 0.060$). Moreover, in multivariate analysis, the overall survival was significantly worse in patients with high TK activity (hazard ratio 34.8, 95% CI 1.03–1176.23; $p = 0.048$). Further, multivariate analysis for prognostic factors among high TK activity and well-established prognostic factors demonstrated that high TK activity was an independent prognostic factor for overall survival (hazard ratio 3.204, 95% CI 1.07–9.62; $p = 0.038$).

Correlations between response with CHOP and prognostic factors

Correlations between response with CHOP and prognostic factors are shown in Table III. The overall response rates among patients with high TK activity and high serum LDH level were 21.4% and 41.7%, respectively. High TK activity and high serum LDH level predicted a poor response ($p = 0.001$ and 0.004). The achievement of a partial response/complete remission with CHOP significantly predicted good survival among all patients with PTCL ($p = 0.000$); median overall survival was 56.4 months (95% CI 27.7–85.2; Figure 1). However, the achievement of a partial response/complete remission with CHOP did not significantly predict good survival among patients with high TK activity and high serum LDH level, respectively ($p = 0.071$ and 0.089).

Correlations between TK activity and other parameters

According to Pearson's product moment correlation coefficient, the closer is the r-value to 1.00, the stronger is the correlation. There was strong correlation between TK activity and sIL-2R (correlation coefficient, $r = 0.795$). There was moderate correlation between TK activity and the following parameters: β2M ($r = 0.382$), LDH ($r = 0.448$), CRP ($r = 0.683$), age ($r = 0.492$), performance status ($r = 0.457$) and histologic subtype ($r = 0.340$). Finally, there was little correlation between TK activity and the following parameters: MIB-1 index ($r = 0.315$), clinical stage ($r = 0.179$) and Hb ($r = 0.170$), respectively.

Discussion

This study suggested that overall survival was significantly worsened in patients with high TK activity, and that the response with CHOP became significantly worse in patients with high TK activity. PTCL is a heterogeneous group of neoplasms that behave aggressively and lead to a poor outcome. For patients with PCTL, it is necessary to use prognostic factors to select the best treatment. In previous studies, reported prognostic factors have included age, Ann Arbor stage, B symptoms, serum LDH level [2–7], serum β2M level [7–9], sIL-2R level [10], MIB-1 index [11–13], IPI [2] and PIT [3].

TK is a cellular enzyme known to be involved in a salvage pathway for DNA synthesis [14]. Mammalian cells contain at least two TK isoenzymes, differing in their biochemical properties and their cellular distribution [15]. TK1 is found in only the G1/S phase of dividing cells [16,17]. TK1 accounts for about 95% of the serum TK activity in most normal and pathologic situations. The mitochondrial isozyme TK2 remains stable throughout the cell cycle. High TK activity has been reported as an aggressive parameter among patients with Hodgkin lymphoma, non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukemia and acute myeloid/lymphoblastic leukemia [18]. Especially in chronic lymphoblastic leukemia and indolent lymphoma, TK activity correlates with the disease stage and provides prognostic information on overall survival and progression-free survival [19–21]. TK activity in chronic lymphocytic leukemia is related to tumor mass and rate of tumor cell proliferation, because the serum TK level correlates with proliferative activity [22]. The proportion of S-phase cells detectable in tumor biopsies has been found to correlate with serum TK level, but not with β2M level [23].

A high serum sIL-2R level is seen in several diseases: malignant neoplasms, autoimmune disorders and infections. Mature T-cells respond to stimulation by antigens, and IL-2 promotes the growth of T-cells bearing IL-2R [24]. Membrane-bound IL-2R is present on all normal activated T-cells [25]. IL-2R consists of three chains: α, β and γ. The α chain appears

Table II. Univariate and multivariate analyses of laboratory parameters that influence overall survival.

Factor	Cut-off	Univariate	Multivariate	Hazard ratio
TK activity	>25 IU/L	0.048	0.048	34.801 (1.030–1176.233)
sIL-2R	>3000 IU/mL	0.06	0.05	0.086 (0.007–0.996)
β2M	>UNL	0.259	0.854	0.785 (0.060–10.342)
LDH	>UNL	0.018	0.314	0.137 (0.003–6.565)
CRP	>UNL	0.013	0.173	3.695 (0.563–24.237)
Anemia		0.642	0.34	6.775 (0.133–343.938)
MIB-1 index	≥30%	0.011	0.101	4.826 (0.737–31.597)

TK, thymidine kinase; sIL-2R, soluble interleukin-2 receptor; β2M, β_2-microglobulin; LDH, lactate dehydrogenase; CRP, C-reactive protein; Hb, hemoglobin; UNL, upper normal limit; LNL, lower normal limit.

RIGHTSLINK

Table III. Relationship of laboratory parameters to complete remission rate and overall response rate with CHOP.

Factor	Median cycles	Complete remission rate (%)	Overall response rate (%)	*p*-Value
All patients	6 (1-9)	34.50	61.80	
TK activity	4 (1-6)	7.10	21.40	0.001
sIL-2R	4.5 (1-9)	10.50	52.60	0.247
β2M	6 (1-9)	26.70	53.30	0.161
LDH	4 (1-9)	20.80	41.70	0.004
CRP	6 (1-9)	33.30	59.20	0.445
MIB-1 index	5 (1-8)	27.20	54.50	0.593

TK, thymidine kinase; sIL-2R, soluble interleukin-2 receptor; β2M, β_2-microglobulin; LDH, lactate dehydrogenase; CRP, C-reactive protein.

on the surface of the T-cell when it is activated, separates from the cell, and exists as a soluble form in the serum. It has been demonstrated that IL-2R is released from the cell surface in a soluble form (sIL-2R) under particular conditions both *in vitro* and *in vivo* [26]. A high serum sIL-2R level reflects an elevated activity of T-cells in the patient's cellular immunity. It is impossible to know whether malignant or normal activated T-cells produce sIL-2R, because both cell types have the capacity to release sIL-2R into the serum. Activated T-cells produce sIL-2R in B-cell and T-cell lymphomas. The serum sIL-2R level reflects the prognosis more obviously in patients with PTCL than B-cell lymphoma because both activated T-cell and lymphoma cells produce sIL-2R in PTCL [10].

A high serum β2M level is known to be an adverse prognostic factor in lymphoproliferative diseases, being directly related to malignant tumor burden [27], but it also keeps its adverse prognostic role when other causes, such as renal dysfunction, are the origin of the raised levels. The β2M level has independent prognostic value in aggressive non-Hodgkin

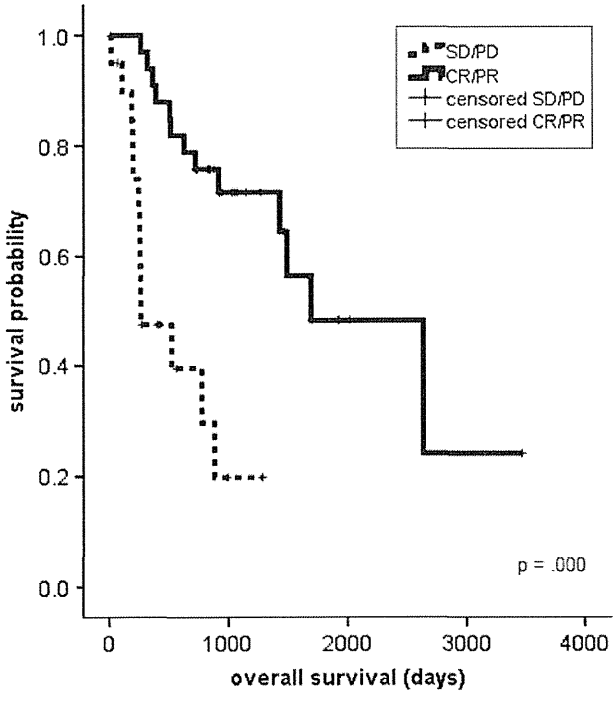

Figure 1. Overall survival curves for all patients with PTCL according to response after CHOP: complete remission plus partial response (CR/PR; $n = 34$) and stable disease plus progressive disease (SD/PD; $n = 20$).

lymphomas [7-9,28]. Moreover, normal active T-cells produce β2M.

Serum LDH represents a surrogate quantitative measure for tumor burden. A high serum LDH level is correlated with the progression of disease in non-Hodgkin lymphoma [29,30]. Combined serum LDH and β2M provide a reliable serologic system for predicting freedom from relapse and survival in large-cell lymphoma. Moreover, elevations in both serum LDH and β2M levels predict shortened remission and survival [7].

CRP is a member of the class of acute-phase reactants, as its level rises dramatically during inflammatory processes occurring in the body. Elevation of serum CRP is due to a rise in the plasma concentration of interleukin-6, which is produced predominantly by macrophages as well as adipocytes. Interleukin-6 is a potent lymphoid growth and differentiation cytokine that is produced by various types of cells, including benign and malignant B and T lymphocytes. Interleukin-6 is implicated in the pathogenesis of several lymphoproliferative disorders, and is reported as an independent prognostic factor for complete remission and failure-free survival among diffuse large-cell lymphomas [31,32]. CRP was reported to be one of the factors strongly correlated with Ann Arbor clinical stage [33].

MIB-1 is alternatively called Ki-67, which is one of the antigens expressed in patients with leukemia. MIB-1 is found throughout the cell cycle, and hence an elevated MIB-1 index shows that lymphoma cells are "in cycle" [8-10]. Cell proliferative fraction as determined by S-phase percentage or immunocytochemistry influences the outcome in both indolent and aggressive lymphomas [13,34]. A high proliferative fraction as defined by expression of the nuclear proliferation antigen Ki-67 identifies patients at risk for early relapse and short survival among those with diffuse large B-cell lymphoma [34].

We demonstrated that TK activity correlated with several parameters. TK activity, sIL-2R, β2M, LDH and CRP are biological parameters that indicate a high tumor burden and proliferative function. On the other hand, there was moderate correlation between TK activity and well-established prognostic factors: age, performance status and histologic subtype. However, TK activity did not correlate strongly with these well-established prognostic factors: three correlation coefficients were less than 0.70.

We also showed that high TK activity significantly influenced overall survival among patients with newly diagnosed PTCL treated with CHOP. Further, high TK activity significantly predicted a poor response with CHOP. Moreover, having a partial response/complete remission with CHOP did not improve the overall survival among patients with PTCL with high TK activity. Thus, we consider that patients with PTCL with high TK activity are relapsed after or refractory to CHOP when more lymphoma cells are in the G1/S phase, because high TK activity correlates with the number of dividing cells in G1/S phase.

The most commonly used regimen is CHOP or its variations. However, while the outcome with CHOP is inadequate, there are few previous data relating to a preferred alternative strategy. In one report, the majority of patients with aggressive

RIGHTSLINK

T-cell lymphoma received a regimen containing anthracycline, but they did not benefit from the use of this regimen over one without anthracycline [1]. More intensive regimens such as ACVBP (adriamycin, cyclophosphamide, vincristine, bleomycin and prednisone) and CyclOBEAP (cyclophosphamide, vincristine, etoposide, bleomycin, adriamycin and prednisone) appear effective against aggressive T-cell lymphomas [35,36].

We consider antimetabolite agents to be effective against PTCL with high TK activity because they attack lymphoma cells specifically in the G1/S phase. HyperCVAD/MA (cyclophosphamide, adriamycin, vincristine and dexamethasone plus cytarabine and methotrexate) has not shown a clear benefit in PTCL retrospectively [37]. However, some groups have reported that gemcitabine-containing regimens such as CHOP-EG (cyclophosphamide, adriamycin, vincristine, prednisone, etoposide and gemcitabine) therapy and GEM-P (gemcitabine, cisplatin and methyprednisolone) therapy, as well as pralatraxate, were effective against T-cell lymphomas [38–41].

In conclusion, high TK activity significantly worsens overall survival among patients with newly diagnosed PTCL treated with CHOP. While we commonly employ CHOP for the treatment of PTCL, we have demonstrated that the overall survival becomes significantly worse in patients who do not achieve a partial response with CHOP. Moreover, the response with CHOP is significantly decreased in patients with high TK activity and does not improve the overall survival among patients with PTCL with high TK activity. The appropriate induction therapy should be selected according to prognostic factors. We suggest that regimens containing antimetabolite agents be employed as induction therapy for patients with PTCL with high TK activity.

Acknowledgements

We thank our attending doctors, dedicated fellows and nurses at the Cancer Institute Hospital, Japanese Foundation for Cancer Research, and we offer special thanks to our patients with lymphoma and their families.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

References

[1] Vose J, Neumann M, Harris M. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcome. J Clin Oncol 2008;26:4124–4130.
[2] A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987–994.
[3] Gallamini A, Stelitano C, Calvi R, et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. Blood 2004;103:2474–2479.
[4] Torizumi K, Aibata H, Yamada R, et al. A study on the measurement of serum thymidine kinase and its clinical significance in hematological neoplastic disorders. Kaku Igaku 1988;26:561–567.
[5] Gerdes J, Lemke H, Baisch H, et al. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984;133:1710–1715.
[6] Gerdes J, Stein H, Pileri S, et al. Prognostic relevance of tumour-cell growth fraction in malignant non-Hodgkin's lymphomas. Lancet 1987;22:448–449.
[7] Swan F Jr, Velaquez WS, Tucker S, et al. A serologic staging system for large-cell lymphomas based on initial beta 2-microglobulin and lactate dehydrogenase levels. J ClinOncol 1989;7:1518–1527.
[8] Johnson PW, Whelan J, Longhurst S, et al. Beta-2 microglobulin: a prognostic factor in diffuse aggressive non-Hodgkin's lymphomas. Br J Cancer 1993;67:792–797.
[9] Jose R, Eulogio C, Antonio G, et al. The adjusted International Prognostic Index and β-2 microglobulin predict the outcome after autologous stem cell transplantation in relapsing/refractory peripheral T-cell lymphoma. Haematologica 2007;92:1067–1074.
[10] Kitagawa J, Hara T, Tsurumi H, et al. Serum-soluble interleukin-2 receptor (sIL-2R) is an extremely strong prognostic factor for patients with peripheral T-cell lymphoma, unspecified (PTCL-U). J Cancer Res Clin Oncol 2009;135:53–59.
[11] Kim SJ, Kim BS, Choi CW, et al. Ki-67 expression is predictive of prognosis in patients with stage I/II extranodal NK/T-cell lymphoma, nasal type. Ann Oncol 2007;18:1382–1387.
[12] Grogan TM, Lippman SM, Spier CM, et al. Independent prognostic significance of a nuclear proliferation antigen in diffuse large cell lymphomas as determined by the monoclonal antibody Ki-67. Blood 1988;71:1157–1160.
[13] Witzig TE, Habermann TM, Kurtin PJ, et al. S-phase fraction by the labeling index as a predictive factor for progression and survival in low grade non-Hodgkin's lymphoma. Cancer 1995;94:1059–1064.
[14] Reichard P, Estborn B. Utilization of deoxyribosides in the synthesis of polynucleotides. J Biol Chem 1951;188:839–846.
[15] Kit S. Viral-associated and induced enzymes. Phamacol Ther 1979;4:501–585.
[16] Bello LJ. Regulation of thymidine kinase synthesis in human cells. Exp Cell Res 1974;89:263–274.
[17] Gronowitz JS, Kallamder CFR, Diderholm H, et al. Application of an in vitro assay for serum thymidine kinase: results on viral disease and malignancies in humans. Int J Cancer 1984;33:5–12.
[18] Hallek M, Wandeders L, Strohmeyer S, Emmerrich B. Thymidine kinase: a tumor marker with prognostic value for non-Hodgkin's lymphoma and a broad range of potential clinical applications. Ann Hematol 1992;65:1–5.
[19] Ellims PH, Eng Gan ET, Medley G, et al. Prognostic relevance of thymidine kinase in adult non-Hodgkin's lymphoma. Blood 1981;58:926–930.
[20] Kallander CF, Simmonsson B, Hagberg H, et al. Serum deoxythymidine kinase gives prognostic information in chronic lymphoblastic leukemia. Cancer 1984;54:2450–2455.
[21] Martinsson U, Glimelius B, Hagberg H, et al. Primarily asymptomatic low grade non-Hodgkin's lymphomas: predication of symptom-free survival and total survival. Eur J Haematol 1989;43:332–338.
[22] Kallander CF, Simonsson B, Gronowitz JS, et al. Serum deoxythymidine kinase correlates with peripheral lymphocyte thymidine uptake in chronic lymphoblastic leukemia. Eur J Haematol 1987;38:331–337.
[23] Lehtinen M, Wigren T, Lehtinen T, et al. Correlation between serum tumor marker levels and tumor proliferation in small cell lung cancer. TumourBiol 1988;9:287–292.
[24] Robb RJ. Human T-cell growth factor: purification and interaction with a cellular receptor. Lymphokine Res 1982;1:37–43.
[25] Cantrell DA, Smith KA. Transient expression of interleukin 2 receptors. Consequences for T cell growth. J Exp Med 1983;158:1895–1911.
[26] Rubin LA, Kurman CC, Fritz ME, et al. Soluble interleukine 2 receptors are released from activated human lymphoid cells in vitro. J Immunol 1985;135:3172–3177.
[27] Hagberg H, Killander A, Simmonsson B. Serum b2-microglobulin in malignant lymphoma. Cancer 1983;51:2220–2225.
[28] Rodriguez J, Cabanillas F, McLaughlin P, et al. A proposal for a simple staging system for intermediate grade lymphoma based on the tumor score. Ann Oncol 1992;3:711–717.
[29] Jagannath S. Tumor burden assessment and its implication for a prognostic model in advanced diffuse large cell lymphoma. J Clin Oncol 1986;4:859–865.
[30] Schneider R. Prognostic significance of serum lactate dehydrogenase in malignant lymphoma. Cancer 1980;46:139–143.
[31] Preti HA, Cabanillas F, Talpaz M, et al. Prognostic value of serum interleukin-6 in diffuse large-cell lymphomas. Ann Intern Med 1997;127:186–194.
[32] Mahmoud FA, Riveca NI. The role of C-reactive protein as a prognostic indicator in advanced cancer. Curr Oncol Rep 2002;4:250–255.

RIGHTSLINK

[33] Perez-Encinas M, Villamayor M, Campos A, et al. Tumor burden and serum level of soluble CD25, CD8, CD23, CD54 and CD44 in non-Hodgkin's lymphoma. Haematologica 1998;83:752–754.
[34] Miller TP, Grogan TM, Dahlberg S, et al. Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas: a prospective Southwest Oncology Group trial. Blood 1994;83:1460–1466.
[35] Tilly H, Lepage E, Coiffer B, et al. Intensive conventional chemotherapy (ACVBP regimen) compared with standard CHOP for poor-prognosis aggressive non-Hodgkin lymphoma. Blood 2003;102:4284–4289.
[36] Niitsu N, Okamoto M, Nakamine H, et al. Clinico-pathologic features and outcome of Japanese patients with peripheral T-cell lymphoma. Hematol Oncol 2008;26:152–158.
[37] Escalon MP, Liu NS, Yang Y, et al. Prognostic factors and treatment of patients with T-cell non Hodgkin lymphoma. Cancer 2005;103:2091–2098.
[38] Zinzani PL, Venturini F, Stefoni V, et al. Gemcitabine as single agent in peripheral T-cell lymphoma patients: evaluation of the long-term outcome. Ann Oncol 2010;21:860–863.
[39] Arkenau HT, Chong G, Cunningham D, et al. Gemcitabine, cisplatin and methyprednisolone for the treatment of patients with peripheral T-cell lymphoma: the Royal Marsden Hospital experience. Haematologica 2007;92:271–272.
[40] Kim JG, Sohn SK, Chea YS, et al. CHOP plus etoposide and gemcitabine (CHOP-EG) as front-line chemotherapy for patients with peripheral T-cell lymphomas. Cancer Chemother Pharmacol 2006;58:35–39.
[41] O'Connor OA, Hamlin PA, Portlock C, et al. Pralatrexate, a novel class of antifol with high affinity for the reduced folate carrier-type 1, produces marked complete and durable remission in a diversity of chemotherapy refractory cases of T-cell lymphoma. Br J Haematol 2007;139:425–428.

RIGHTSLINK

Japanese Journal of Clinical Oncology Advance Access published June 13, 2012

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2012
doi:10.1093/jjco/hys085

Original Article

Feasibility and Efficacy of Combined Cisplatin and Irinotecan Chemotherapy for Poorly Differentiated Neuroendocrine Carcinomas

Kenji Nakano[1], Shunji Takahashi[1,*], Takeshi Yuasa[1,2], Noriko Nishimura[1], Yuko Mishima[1], Sakura Sakajiri[1], Masahiro Yokoyama[1], Naoko Tsuyama[3], Yuichi Ishikawa[3] and Kiyohiko Hatake[1]

[1]Department of Hematology and Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, [2]Department of Urology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake and [3]Division of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan

*For reprints and all correspondence: Shunji Takahashi, Department of Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo 135-8550, Japan. E-mail; stakahas@jfcr.or.jp

Received March 8, 2012; accepted May 11, 2012

Objective: No standard treatment has been established for poorly differentiated neuroendocrine carcinoma; the usual recommended treatment is based on the strategy for small cell lung carcinoma. The aim of this study was to evaluate the response of poorly differentiated neuroendocrine carcinoma to the combination of irinotecan and cisplatin in one institution.
Methods: We retrospectively reviewed 50 poorly differentiated neuroendocrine carcinoma patients treated from September 2005 to April 2011 in our institution. Patients were divided into two stages: limited disease or extensive disease. Forty-four patients received the combination chemotherapy of irinotecan and cisplatin, consisting of 4-week cycles of 60 mg/m^2 irinotecan on days 1, 8, 15 and 60 mg/m^2 cisplatin on day 1.
Results and conclusion: Median age was 60 years. Median follow-up time was 11.4 months. Overall survival did not reach the median, and 1-year overall survival was 67%. The response rate was 50% (64% at first line), and progression-free survival was 4.8 months (7.3 months at first line). Grade 3–4 hematologic adverse events were seen in 29 patients (66%) and Grade 3–4 non-hematologic adverse events were seen in 20 patients (45%), but no patients died of adverse events. Multivariate analysis showed a statistically significant relationship with neuron-specific enolase elevation and poor overall survival ($P = 0.016$, hazard ratio 6.261, 95% confidence interval). The combination chemotherapy of irinotecan and cisplatin is moderately effective and feasible, and it should be considered as a treatment option for poorly differentiated neuroendocrine carcinoma.

Key words: neuroendocrine carcinoma – extrapulmonary small cell carcinoma – irinotecan – cisplatin

INTRODUCTION

Neuroendocrine carcinomas are components of neoplasms that have immunohistochemical staining characteristics (chromogranin A, synaptophysin and CD56/NCAM) or ultrastructural features (neurosecretory granules). Prognoses of neuroendocrine carcinoma patients are various, and for some of them, especially poorly differentiated neuroendocrine carcinoma (PDNEC) patients, it is poor. PDNEC arises from almost all organs and some components are similar to small cell lung carcinoma (SCLC) in morphology and are called extrapulmonary small cell carcinoma (EPSCC). EPSCC was first described in 1930 by Duguid and Kennedy (1) and accounts for 0.1–0.4% of all malignancies, and 5% of all small cell carcinomas (2,3). In lung carcinoma, large cell carcinomas with neuroendocrine characteristics are called

© The Author 2012. Published by Oxford University Press. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

large cell neuroendocrine carcinoma (LCNEC) and LCNEC patients have recently been known to have as poor prognoses as SCLC patients (4). Other than lung carcinoma, in clinical practice, PDNEC and EPSCC are usually treated in the same way. Because of their rarity, however, there has not been enough clinical data to establish a treatment strategy for PDNEC patients. The usual recommendation is to use the same treatment strategy as for SCLC.

For a long time, the standard systemic chemotherapy for advanced SCLC has been a combination of cisplatin (CDDP) and etoposide (EP regimen) (5,6). Recently, combined cisplatin and irinotecan (IP regimen) was reported to be as effective as the EP regimen for SCLC. The aim of this study was to evaluate the response of PDNEC to the combination of IP regimen in one institution.

PATIENTS AND METHODS

Patients

We retrospectively evaluated the clinical courses of PDNEC patients diagnosed and treated between September 2005 and April 2011 in the Cancer Institute Hospital of Japanese Foundation for Cancer Research, Tokyo.

All patients were diagnosed pathologically. Pathological diagnosis of PDNEC was based on the 2004 World Health Organization criteria for lung cancer (7), including criteria for SCLC and LCNEC. In order to assist the pathological diagnosis, immunohistochemistry was performed using the antibodies for neuroendocrine markers including synaptophysin, chromogranin A and NCAM/CD56. At least one of the neuroendocrine markers had to be positive in diagnosing PDNEC. Patients with well-differentiated neuroendocrine tumors (or carcinoids), Merkel cell tumors, or patients diagnosed with a lung primary (SCLC or LCNEC) clinically were excluded in the current analysis. The primary organ was evaluated based on both radiological imaging and immunohistochemistry. Patients with a lung lesion on chest computed tomography (CT) at diagnosis and positive thyroid transcription factor-1 (TTF-1) in the specimens who were diagnosed as lung primary were excluded.

Patients were staged by utilizing a two-stage system based on that of SCLC (8). Limited disease (LD) was defined as a tumor localized to the organ of origin and the locoregional lymph nodes that could easily be encompassed within one radiation therapy (RT) treatment portal. Extensive disease (ED) was defined as a tumor spreading beyond one radiation portal or with any metastatic lesion.

Treatment Strategies

The CDDP/ IP regimen consisted of 60 mg/m^2 IP on days 1, 8, 15 and 60 mg/m^2 CDDP on day 1 every 4 weeks, with adequate hydration and antiemetic drugs as for SCLC studies (4). On day 8 or 15, if severe hematologic or non-hematologic toxicities were present, IP was not administered. If the leukocyte level fell below 2000/mm^2 or the neutrophil level fell below 1000/mm^2, recombinant human granulocyte colony-stimulating factor was administered until the leukocyte or neutrophil count was restored.

The IP regimen was repeated until disease progression, patient refusal or unacceptable toxicity occurred. If Grade 3–4 non-hematological toxicity or prolonged Grade 4 hematological toxicity occurred, the dose of CDDP and IP was reduced to 80%. After completion, additional locoregional therapy or palliative therapies were performed if appropriate. Prophylactic cranial irradiation was not performed.

Evaluation

For measurable disease, responses were evaluated using CT and magnetic resonance imaging according to the response evaluation criteria in solid tumors, version 1.1. The National Center Institute Common Terminology Criteria for Adverse Events (version 4.0) was used to evaluate toxicity. Using the Kaplan–Meier method, the progression-free survival (PFS) and overall survival (OS) were calculated from the start of the IP regimen to the disease progression and death, respectively. Prognostic factors for OS were compared using a log-rank test and the Cox proportional hazards model (9,10). All statistical tests were two sided.

RESULTS

Patients

Between September 2005 and April 2011, 50 patients were diagnosed with PDNEC in the study institution and 44 were treated with the IP regimen. Six patients never received IP chemotherapy in their clinical courses. Of those, two patients with LD stage of head and neck origin were treated with concurrent chemoradiotherapy with CDDP, according to the treatment strategy for squamous cell head and neck carcinoma (11). Two patients were treated with multimodal therapy including surgery, radiotherapy and chemotherapy other than the IP regimen. The remaining two patients died without receiving any therapy because of aggressive progression.

There were 26 males and 18 females. The median age at diagnosis was 60 years (range: 26–80). The primary sites were as follows: 9 gastrointestinal, 18 head and neck, 4 urinary tract, 1 gynecologic organ and 12 unknown primary origins. Eight patients had lung metastases, 15 patients had bone metastases and 13 patients had liver metastases at diagnosis. Fourteen patients had LD, whereas 30 had ED. Although these 14 LD patients were considered for locoregional therapy before the IP regimen, neither the surgical treatment nor the curative radiation therapy was performed because of local invasion. All these patient characteristics are summarized in Table 1.

Table 1. Patient characteristics

Characteristics	No.	(%)
Age		
Median	60	
Range	26–80	
Gender		
Male	26	59
Female	18	41
Smoking		
Smoker	20	45
Non-smoker	19	43
Unknown	5	11
Clinical stage		
Limited disease	14	32
Extensive disease	30	68
Performance status		
0	24	54
1	11	25
≥2	2	5
Unknown	7	16
Pre-treatment		
Previously untreated	28	64
Surgery	7	16
Radiotherapy	7	16
Chemotherapy	9	20
Primary site		
Gastrointestinal tract	9	20
Head and neck	18	41
Urinary tract	4	9
Gynecologic organ	1	2
Unknown origin	12	28
Metastatic site		
Lung	8	18
Bone	15	34
Liver	13	30
Serum tumor marker		
NSE (>UNL)	31	70
NSE (≤UNL)	11	25
Not done	3	7
Pro-GRP (>UNL)	12	27
Pro-GRP (≤UNL)	20	45
Not done	12	27
Pathological marker		
Synaptophysin +	39	89
Synaptophysin −	4	9
Not done	1	2
Chromogranin A +	30	68
Chromogranin A −	11	25
Not done	3	7
CD56/NCAM +	37	84
CD56/NCAM −	5	11
Not done	2	5
TTF-1 +	8	18
TTF-1 −	28	64
Not done	8	18
Total	44	

NSE, neuron-specific enolase; TTF-1, thyroid transcription factor-1.

Immunohistochemical Results for PDNEC

Synaptophysin was evaluated in 43 patients and shown to be positive in 39 patients (91%), chromogranin A was positive in 30 patients out of 41 patients evaluated (73%) and CD56/NCAM was positive in 37 patients out of 42 patients evaluated (88%). TTF-1 was also evaluated in 36 patients and tumors of 8 patients were positive. TTF-1 is usually used for a pathological marker of primary lung cancer, but all of the eight patients with TTF-1 immunoreactivity proved to have no lung lesions closely examined by chest CT at diagnosis. Therefore, they were regarded as extrapulmonary tumors clinically.

Treatment

Forty-four patients received chemotherapy with the IP regimen. Among these patients, 28 patients were treated with IP as a first-line therapy, whereas 16 patients received one or more therapies (surgery, radiation therapy and/or other chemotherapy regimens) before IP. As for chemotherapy, 35 patients were chemo-naïve and 9 patients had a history of systemic chemotherapy. After the IP regimen, 30 patients received other curative or palliative therapies, including radiotherapy for 15 patients, surgical operation for 5 and salvage chemotherapy for 18. There were 16 patients who received therapies with curative intent after the IP regimen, radiotherapy for 11, surgical operation for 3 and other chemotherapy for 3 patients; 5 patients relapsed after curative therapies. In 14 LD patients, 8 patients received curative therapies and 3 patients relapsed. The other 14 patients received palliative therapies after progression. Salvage chemotherapy regimens consisted of IP monotherapy, amrubicin, S-1, combined carboplatin and paclitaxel, and combined cisplatin and EP.

The Response and Outcome of the Patients who Underwent IP Regimen

At the time of analysis, 30 patients had progressed after the IP regimen and 19 patients had died of disease. Median treatment cycles of IP regimen were 3 (range: 1–8). The overall response rate to the IP regimen was 50%, with 3 patients (7%) achieving complete response (CR; Table 2). In patients receiving the IP regimen as first-line therapy, the response rate was 64%, whereas in patients receiving other therapies before IP regimen, the response rate was 25%.

The median follow-up time was 11.4 months (range: 1.2–46.9 months). The median PFS of the IP regimen chemotherapy was 4.8, and median OS was not reached (Fig. 1A and B). The 1-year, 2-year and 3-year survival rates were 67, 42 and 21%, respectively. Although LD patients tended to have a better PFS and OS, there was no statistical significance for either of them (Fig. 1C and D). Median PFS for patients receiving the IP regimen as first-line chemotherapy was 7.3 months, whereas the median PFS was 3.6 months for patients who had already received any other chemotherapy before the IP regimen; there was a significant difference ($P = 0.003$). However, no statistical significance was shown for the OS by the log-rank test ($P = 0.848$; Fig. 2A and B). No significant difference was seen among the primary organs of PDNEC.

Toxicity

The major adverse events of IP therapy are shown in Table 3. Grade 3 or 4 hematological adverse events were seen in 29 patients (66%), most of them being leukocytopenia or neutropenia. Grade 3 or 4 non-hematological adverse events were seen in 20 patients (45%). Hyponatremia was the most frequently seen severe non-hematological adverse event (18%). Although 18 patients (41%) needed to reduce the treatment dose and 2 patients had to discontinue the IP due to adverse events, no therapy-related death was seen in this treatment period. One patient discontinued the IP regimen because of a Grade 2 skin eruption that occurred after every infusion of IP. The other patient suffered from febrile neutropenia and septic shock during the first course of the IP regimen.

Prognostic Factor

Finally, we examined the prognostic factors of the IP regimen for these patients. Old age (>60 year old), poor ECOG PS (>1), ED stage, presence of prior chemotherapy, post-chemotherapy, presence of particular lesions (lung, liver and bone) and elevation of serum tumor markers [neuron-specific enolase (NSE) and Pro-GRP] were evaluated in the univariate analysis. Of them, old age and NSE elevation were shown to be prognostic factors for poor OS ($P = 0.031$ and 0.012, respectively, Fig. 2C and D). Multivariate analysis also showed a statistically significant relationship with elevation of serum NSE level and poor OS ($P = 0.016$, hazard ratio, 6.261, 95% CI: 1.400–27.998).

Table 2. Response of the irinotecan (IP) regimen

Patients	No.	Outcome				
		OS (months)	PFS (months)	OR (%)	CR (%)	PR (%)
All patients	44	16	4.8	22 (50)	3 (7)	19 (43)
Clinical stage						
Limited disease	14	Not reached	10.7	8 (57)	3 (21)	5 (36)
Extensive disease	30	14.3	4.5	14 (46)	0 (0)	14 (46)
P value (log-rank)		0.237	0.185			
Previous therapy						
Previous therapy −	28	16	6.4	18 (64)	2 (7)	16 (57)
Previous therapy +	16	14.6	4.2	4 (25)	1 (6)	3 (19)
P value (log-rank)		0.85	0.041			
Primary site						
Gastrointestinal tract	9	Not reached	5.1	4 (44)	1 (11)	3 (33)
Head and neck	18	Not reached	4.2	8 (44)	1 (6)	7 (39)
Urinary tract	4	12.5	1.4	1 (25)	0 (0)	1 (25)
Gynecologic organ	1	16	6.4	0 (0)	0 (0)	0 (0)
Unknown origin	12	14.3	10.6	9 (75)	1 (8)	8 (67)
P value (log-rank)		0.812	0.055			

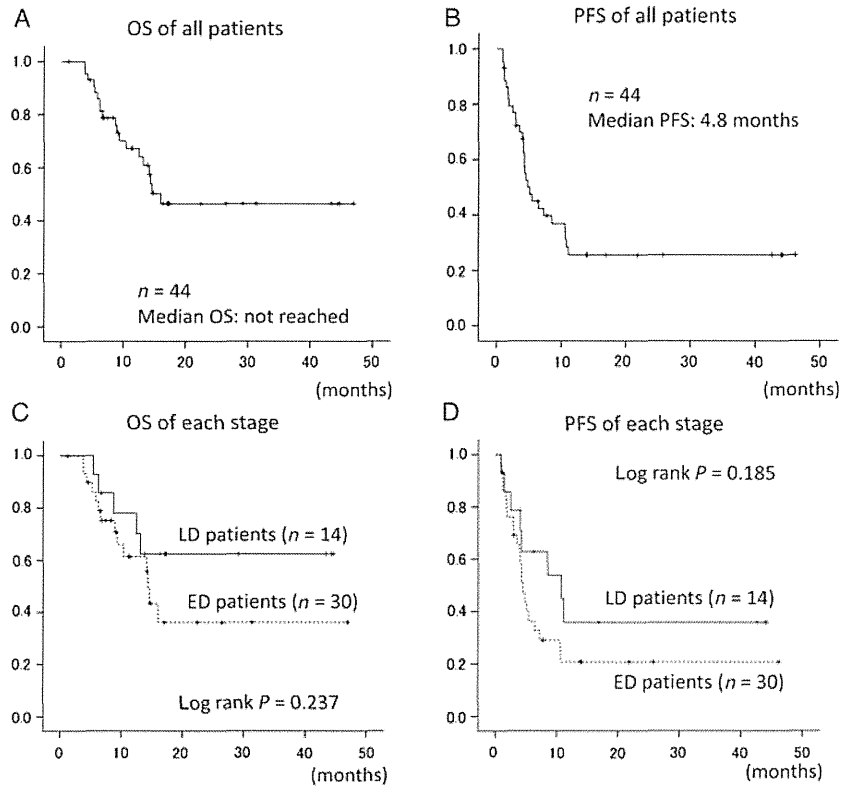

Figure 1. Prognoses of patients.

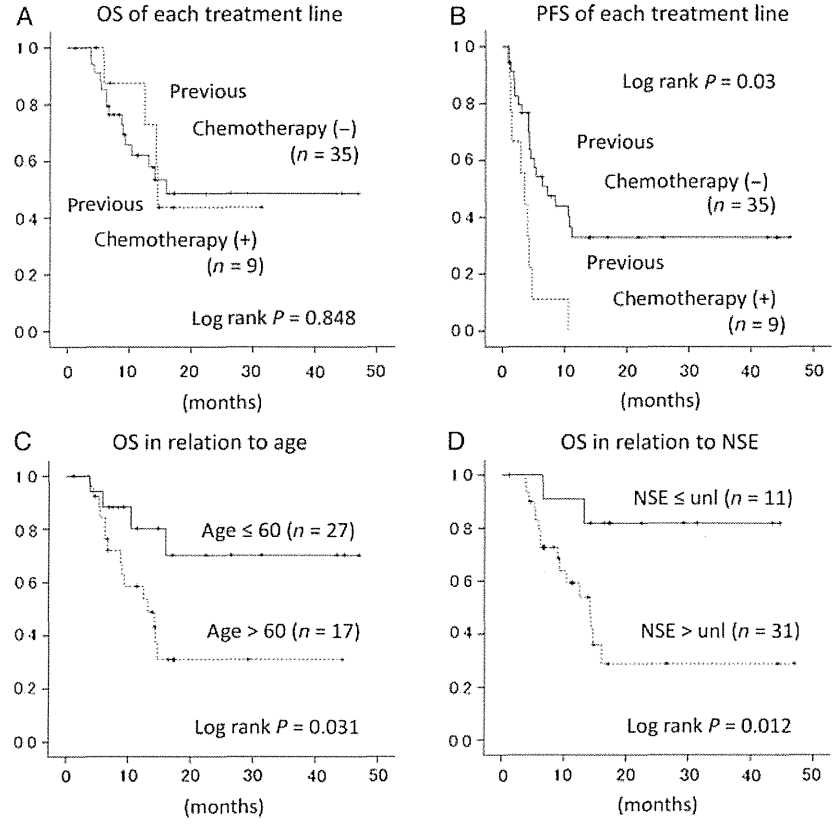

Figure 2. Prognostic factors.

Table 3. Toxicities of the IP regimen

Adverse events	All grade		Grade 3		Grade 4	
	No.	%	No.	%	No.	%
Hematologic adverse events						
Leukocytopenia	39	89	14	32	5	11
Neutropenia	38	86	15	34	11	25
Anemia	40	91	8	18	3	7
Thrombocytopenia	5	11	0	0	2	5
Non-hematologic adverse events						
Nausea	33	75	2	5	0	0
Vomiting	14	32	1	2	0	0
Diarrhea	24	55	4	9	0	0
Constipation	18	41	0	0	0	0
Infection	9	20	6	14	0	0
AST elevation	25	57	1	2	0	0
ALT elevation	21	48	1	2	0	0
T-Bil elevation	9	20	1	2	0	0
Creatinine elevation	14	32	0	0	0	0
Hyponatremia	18	41	5	11	3	7
Hyperkalemia	11	25	1	2	0	0
Peripheral neuropathy	6	14	0	0	0	0
Other non-hematologic AE[a]	14	32	3	7	0	0

[a]Other non-hematologic AE: tumor lysis syndrome G3 (1), vertigo G3 (1), sick sinus syndrome G3 (1).

DISCUSSION

Until now, no standard chemotherapeutic regimen has been established for PDNEC. In this study, our retrospective analysis demonstrated that the IP regimen was moderately effective and feasible, suggesting that the IP regimen could be considered as a treatment option for PDNEC. Noda et al. (5) reported from the prospective clinical trial for the ED SCLC patients that the IP regimen showed a better response rate and a more improved prognosis than the EP regimen. However, after that, Hanna et al. (6) again compared the IP regimen with EP in a randomized phase 3 study, which showed that the IP regimen and EP regimens had almost the same response, although the doses and schedules used were different from the previous study. Recently, Jin et al. (12) reported their clinical experience with cisplatin and IP for 15 EPSCC patients, although the treatment schedule was different. In our study, the treatment schedule was the same as that of Noda's study.

In general, ED-stage patients have a very poor prognosis. Haider et al. (13) reported on the prognoses of 101 EPSCC patients diagnosed in Saskatchewan, Canada, from 1971 to 2002. In their study, the median OS of LD patients was 34 months, much longer than the median OS of ED patients, which was only 2 months. Moreover, ED-stage PDNEC patients with unknown primary origin have very poor prognoses. Though some case reports showed a long survival of LD-stage PDNEC patients with unknown primary origin, median survivals of ED-stage patients with unknown primary have been less than 1 year (14).

In retrospective studies, a platinum-based regimen and a doxorubicin-based regimen were used for PDNEC patients and they showed moderate responses, but long-term prognoses are poor (12,15–17). Hainsworth et al. (17) reported moderate response and OS with a three-drug chemotherapy regimen (paclitaxel, carboplatin and EP), and our study has as same response and survival rate as Hainsworth's study with a two-drug regimen. In our study, two-thirds of our patients were ED stage, and PDNEC patients with unknown primary origin accounted for 24%. The IP regimen is useful for the ED stage, and/or unknown primary PDNEC patients.

In our study, high serum NSE was shown to be a prognostic factor of poor OS in PDNEC patients receiving the IP regimen. Serum NSE is known to be a tumor marker of SCLC, and some investigators have suggested that NSE is related to the prognosis for SCLC. Shibayama et al. (18) evaluated the usefulness of Pro-GRP and NSE for the diagnosis and prognosis of SCLC and in the SCLC patients receiving chemotherapy. CR rate in patients with elevated NSE levels was significantly lower than in patients with normal levels of NSE (18.5% vs. 61.7%, $P < 0.001$). However, whether NSE is related to the prognosis of PDNEC patients has not yet been evaluated. In earlier studies, Lin et al. (19) reported favorable prognostic factors of PDNEC, based on a retrospective analysis of 90 PDNEC patients; female gender, LD and combined modality treatment are discussed in the article. We found no significant difference in the prognoses of patients based on gender or stage. We need to collect and compare more information about the clinical features and prognoses of PDNEC patients to identify the best prognostic factors.

In conclusion, the IP regimen can be considered as a good treatment option for PDNEC patients, especially patients with unknown primary and ED stage. However, we note that the response rate and PFS were relatively poor in previously treated patients. We should pursue a new salvage treatment option for refractory and relapsed PDNEC in the future.

Funding

This work was partly supported by the Takeda Science Foundation, and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Conflict of interest statement

None declared.

References

1. Duguid J, Kennedy A. Oat cell tumors of mediastinal glands. *J Pathol Bacteriol* 1930;33:93–9.
2. Remick SC, Hafez GR, Carbone PP. Extrapulmonary small cell carcinoma. A review of the literature with emphasis on therapy and outcome. *Medicine* 1987;66:457–61.
3. Remick SC, Ruckdeschel JC. Extrapulmonary and pulmonary small-cell carcinoma: tumor biology, therapy, and outcome. *Med Pediatr Oncol* 1992;20:89–99.
4. Asamura H, Kameya T, Matsuno Y, et al. Neuroendocrine neoplasms of the lung: a prognostic spectrum. *J Clin Oncol* 2006;24:70–6.
5. Noda K, Nishiwaki Y, Kawahara M, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* 2002;346:85–91.
6. Hanna N, Bunn PA, Jr, Langer C, et al. Randomized phase III trial comparing irinotecan/cisplatin with etoposide/cisplatin in patients with previously untreated extensive-stage disease small-cell lung cancer. *J Clin Oncol* 2006;24:2038–43.
7. Travis WD, Brambilla E, Muller-Hermelink HK, Harris CC. In: World Health Organization Classification of Tumours, editor. *Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart*, Vol 10. Lyon, France: IARC Press 2004.
8. Clark R, Ihde DC. Small-cell lung cancer: treatment progress and prospects. *Oncology* 1998;12:47–58.
9. Kaplan EL, Meider P. Non parametric estimation from incomplete observations. *J Am Stat Assoc* 1958;53:457–81.
10. Sauerbrei W, Hollander N, Bucholz A. Investigation about a screening step in model selection. *Stat Comput* 2008;18:195–208.
11. Adelstein DJ, Li Y, Adams GL, et al. An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. *J Clin Oncol* 2003;21:92–8.
12. Jin S, Wang T, Chen X, et al. Phase II study of weekly irinotecan plus cisplatin with previously untreated extensive-stage extrapulmonary small cell carcinoma. *Onkologie* 2011;34:378–81.
13. Haider K, Shahid RK, Finch D, et al. Extrapulmonary small cell cancer: a Canadian province's experience. *Cancer* 2006;107:2262–9.
14. Lobins R, Floyed J. Small cell carcinoma of unknown primary. *Semin Oncol* 2007;34:39–42.
15. Galanis E, Frytak S, Lloyd RV. Extrapulmonary small cell carcinoma. *Cancer* 1997;79:1729–36.
16. Van Der Gaast A, Verwey J, Prins E, Splinter TA. Chemotherapy as treatment of choice in extrapulmonary undifferentiated small cell carcinoma. *Cancer* 1990;65:422.
17. Hainsworth JD, Spigel DR, Litchy S, Greco FA. Phase II trial of paclitaxel, carboplatin, and etoposie in advanced poorly differentiated neuroendocrine carcinoma: a Minnie Pearl Cancer research Network Study. *J Clin Oncol* 2006;24:3548–54.
18. Shibayama T, Ueoka H, Nishi K, et al. Complementary roles of pro-gastrin-releasing peptide (ProGRP) and neuron specific enolase (NSE) in diagnosis and prognosis of small-cell lung cancer (SCLC). *Lung Cancer* 2001;32:61–9.
19. Lin YL, Chung CY, Chang CS, et al. Prognostic factors in extrapulmonary small cell carcinomas. A large retrospective study. *Oncology* 2007;72:181–7.

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2013;43(1)37–44
doi:10.1093/jjco/hys194
Advance Access Publication 19 November 2012

Prognostic Value of C-reactive Protein, Lactase Dehydrogenase and Anemia in Recurrent or Refractory Aggressive Lymphoma

Kazuhito Suzuki*, Yasuhito Terui, Noriko Nishimura, Yuko Mishima, Sakura Sakajiri, Masahiro Yokoyama, Shunji Takahashi, Naoko Tsuyama, Kengo Takeuchi and Kiyohiko Hatake

Department of Medical Hematology/Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Tokyo, Japan

*For reprints and all correspondence: Kazuhito Suzuki, Department of Medical Hematology/Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 1358550, Japan. E-mail: kazuhito.suzuki@jfcr.or.jp

Received May 25, 2012; accepted October 22, 2012

Objective: Prognostic predictors for newly diagnosed malignant lymphoma are well known. However, they have not been compared for patients with recurrent or refractory malignant lymphoma.

Methods: We retrospectively analyzed biological prognostic predictors for patients with recurrent or refractory aggressive lymphoma, such as serum levels of C-reactive protein, lactate dehydrogenase, hemoglobin, β2-microglobulin and soluble interleukin-2 receptor before salvage therapy. The primary endpoint was overall survival after salvage treatment. First, univariate and multivariate analyses were performed for each of the parameters, using the log-rank test and Cox regression analysis, respectively. Secondly, we classified the patients into three risk groups on the basis of significant poor predictors.

Results: Sixty-three patients, including 41 patients with diffuse large B-cell lymphoma, were included in this study. Overall survival was significantly worse in patients with elevated C-reactive protein level (hazard ratio 3.757; $P = 0.017$), elevated lactate dehydrogenase level (hazard ratio 3.948; $P = 0.010$) and anemia (hazard ratio 3.925; $P = 0.016$) by multivariate analysis. We classified patients into two groups based on these three biological parameters. The median overall survival of the high- and low-risk patients was 5.8 and 60.1 months, respectively (log-rank test; $P < 0.001$). The overall response rate was significantly higher among the low-risk patients than among the high-risk patients (71.4 versus 28.6%, $P = 0.005$). Those results were similar among all aggressive lymphoma and diffuse large B-cell lymphoma.

Conclusions: Elevated C-reactive protein level, elevated lactate dehydrogenase level and anemia before salvage treatment predicted poorer outcomes among patients with recurrent or refractory aggressive lymphoma.

Key words: aggressive lymphoma – prognosis – C-reactive protein – lactase dehydrogenase – anemia

INTRODUCTION

For patients who have experienced recurrence or are refractory to induction therapy, we have the choice of salvage chemotherapies such as ICE (1), DHAP (2), ESHAP (3) and so on. High-dose chemotherapy followed by autologous hematopoietic stem cell transplantation is the treatment of choice for patients with recurrent or relapsed aggressive lymphoma who still respond to salvage therapy (4,5). Recently, the

© The Author 2012. Published by Oxford University Press. All rights reserved.
For Permissions, please email: journals.permissions@oup.com

Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) study published a study comparing the effects of two well-established salvage regimens, R-ICE and R-DHAP, followed by autologous hematopoietic stem cell transplantation for relapsed and large B-cell lymphoma in the Rituximab era (6).

Prognostic predictors for newly diagnosed malignant lymphoma, such as the International Prognostic Index (IPI) (7), Revised IPI (R-IPI) (8), Follicular Lymphoma IPI (FLIPI) (9), Prognostic Index for Peripheral T-cell Lymphoma (PIT) (10) and International Prognostic Score (IPS) (11), are well known. Moreover, several prognostic factors for the outcome of autologous hematopoietic stem cell transplantation have been described in the literature: chemotherapy sensitivity before autologous hematopoietic stem cell transplantation (12), time from diagnosis to relapse of <12 months (13) and the presence of prognostic factors at relapse, as defined by the Secondary Age-Adjusted IPI (saaIPI) (14,15). On the other hand, prognostic factors have not been compared for patients who are eligible and ineligible for autologous hematopoietic stem cell transplantation, with recurrent or refractory malignant lymphoma.

The purpose of this study was to investigate laboratory prognostic factors, including serum C-reactive protein (CRP) level, serum lactate dehydrogenase (LDH) level, hemoglobin concentration (Hb), serum β2-microglobulin (β2M) level and serum soluble interleukin-2 receptor (sIL-2R) level before salvage therapy, in order to evaluate them for overall survival (OS) among patients with recurrent or relapsed aggressive lymphoma treated with ICE or DHAP with or without rituximab. All of the patients were enrolled in a retrospective single-center study at the Cancer Institute Hospital, Japanese Foundation for Cancer Research.

METHODS

Patients

We retrospectively analyzed patients with recurrent or refractory malignant lymphoma treated with ICE or DHAP with or without rituximab as salvage treatment in our institute from April 2005 to June 2010. All the patients received ICE and DHAP as the first salvage therapy. Patients were analyzed if they were older than 18 years with aggressive lymphoma. Patients were excluded if they had clinically relevant cardiac disease or positivity for antibodies against HIV-1 or -2. We define recurrent and refractory disease by Cheson's criteria in 1999 (16). They included the following subtypes: 41 diffuse large B-cell lymphoma, 8 peripheral T-cell lymphoma not otherwise specified, 6 angioimmunoblastic T-cell lymphoma, 4 extranodal NK/T lymphoma, nasal-type, 2 anaplastic lymphoma kinase-negative anaplastic large-cell lymphoma and 2 follicular lymphoma, grade 3b. Two patients was transformed B-cell lymphoma from follicular lymphoma, and extranodal marzinal zone lymphoma of mucosa-associated lymphoid tissue. All pathological specimens were diagnosed by hematopathologists in our institute. This study was approved by the Independent Ethics Committees/Institutional Review Boards in our institute. Patients provided written informed consent before entering the study, which was carried out in accordance with the Declaration of Helsinki.

Treatment and Assessment of Response

The patients received ICE (ifosfamide, 1700 mg/m^2, Days 1–3; carboplatin, area under the curve = 5, Day 1; etoposide, 100 mg/m^2, Days 1–3) or DHAP (dexamethasone, 40 mg/body, Days 1–4; cytarabine, 2 g/m^2, Day 2; cisplatin, 100 mg/m^2, Day 1) as a salvage therapy. The dose of cytarabine is approved up to 2 g/m^2 in Japan. We added rituximab (375 mg/m^2) when CD20 was positive histologically. Patients who were 65 years or younger without coexisting comorbidities and who had achieved complete remission or a partial response after three cycles of salvage therapy received high MEC (carboplatin, 320 mg/m^2, Days 1–4; etoposide, 160 mg/m^2, Days 1–4; melphalan, 70 mg/m^2, Days 5–6). Peripheral blood stem cells were infused on Day 0, at least 24 h after completion of high MEC. Patients who did not achieve complete response or partial response after the salvage therapy were approved to receive other salvage therapies, such as ICE, DHAP, hyper-CVAD (cyclophosphamide, vincristine, adriamycin, dexamethasone, methotrexate and cytarabine), low-dose etoposide, irinotecan and allogeneic hematopoietic stem cell transplantation.

Evaluations were carried out on the following criteria. Complete remission was defined as the disappearance of all clinical evidence of lymphoma, with no persisting disease-related symptoms. Partial response was defined as a decrease $>50\%$ in the sum of the products of the two longest diameters of all measurable lesions and non-measurable lesions had to decrease by at least 50%. Progressive disease was defined as any increase $>50\%$ in the sum of the diameters of any measurable lesions or the appearance of new lesions. Stable disease was considered to be any condition intermediate between partial response and progressive disease (16).

Prognostic Factors

The following parameters were recorded and evaluated for prognosis: serum CRP level, serum LDH level, Hb, serum β2M level and serum sIL-2R level before salvage therapy. The cut-offs of CRP, LDH and β2M levels were defined with upper normal limits, and that of Hb was defined with a lower normal limit. The cut-off of sIL-2R was defined as 1000 IU/l. Moreover, we analyzed other, well-established prognostic factors: age >60 years, stage of the disease beyond II, performance status >1, IPI >2 and interval from induction therapy to salvage therapy within 1 year. The disease stage was determined according to the Ann Arbor classification, and the performance status was determined

according to the Eastern Cooperative Oncology Group (ECOG) score.

Statistical Analysis

The primary endpoint was to evaluate laboratory parameters that would predict poor OS after salvage treatments were started. First, univariate analysis was performed for each of the parameters indicated above. Actuarial survival analysis was performed by the method described by Kaplan and Meier, and the curves were compared by the log-rank test. All prognostic variables were considered for the multivariate analysis for survival. The latter was performed by Cox regression analysis. Secondly, we classified the patients into three risk groups due to significant poor predictors from the laboratory parameters. Actuarial survival analysis among the two risk groups was performed by the method described by Kaplan and Meier, and the curves were compared by log-rank test. Moreover, we analyzed prognostic factors between significant poor predictors from the laboratory parameters and well-established prognostic factors, such as disease status, performance status, international index and interval from induction therapy to salvage therapy, by Cox regression analysis. Secondly, correlations between partial response plus complete remission rate by salvage therapies and the laboratory parameters were assessed by the χ^2 test and Fisher's exact test. Finally, we analyzed the correlation between the five laboratory parameters by Pearson's product–moment correlation coefficient in order to know whether the significant risk factors predicted poor outcome independently. All *P*-values reported were two-sided, and statistical significance was defined as $P < 0.05$. The statistical analyses were performed with the SPSS statistical software (SPSS, Inc., Chicago, IL, USA).

RESULTS

Patients and Treatment

Sixty-three patients with recurrent or refractory malignant lymphoma were enrolled into this study; of which, 46 had recurrent lymphoma and 17 had refractory lymphoma. The main patient characteristics are shown in Table 1. The median age of the patients in this study was 59.5 years (range, 26.9–76.1 years). As induction therapy, 39 received R-CHOP, 16 had CHOP, 3 had EPOCH, 2 had DeVIC plus radiation therapy, 1 had high-CHOP, 1 had radiation therapy and 1 was unknown. The levels of laboratory factors are shown in Table 2. The median levels of CRP, LDH, Hb, β2M and sIL-2R were 0.3 IU/l (range, 0.1–26.7 IU/l), 258 IU/l (range, 130–6510 IU/l), 11.8 g/l (5.0–15.8 g/l), 2.08 IU/l (range, 1.21–4.44 IU/l) and 984 IU/l (range, 296–7840 IU/l), respectively. Fifty and 13 patients received ICE and DHAP as a salvage therapy, respectively. The median number of cycles of ICE and DHAP were three (range, 1–4) and two (range, 1–3), respectively.

Table 1. Patient characteristics

	Patients ($n = 63$)	
	Number	Percentage
Age	59.5 (26.9–76.1) year	
Gender		
Male	38	60.3
Female	25	39.7
Performance status		
0, 1	45	88.2
2, 3, 4	6	11.8
Histological subtypes		
DLBCL	41	65.1
Non-DLBCL	22	34.9
Ann Arbor clinical stage		
I/II	20	31.7
III/IV	43	68.3
IPI		
0,1,2	30	51.8
3,4,5	28	48.2
Induction therapy		
CHOP ± R	55	87.3
Others	8	12.7
Interval from induction therapy to salvage therapy		
Under 1 year[a]	38	60.3
Above 1 year	25	39.7

DLBCL, diffuse large B-cell lymphoma; IPI, International Prognostic Index score; CHOP, cyclophosphamide, doxorbicin, vincristine and predonisone; R, rituximab; ABVD, doxorubicin, bleomycin, vinbrastine and dacarbazine.
[a]Including patients not achieving complete response after the first-line therapy.

Table 2. Laboratory factors

Factors	Median level	Cut-off	Number	Percent
CRP	0.3 (0.1–26.7) IU/l	>unl	25	39.7
		≤unl	38	60.3
LDH	258 (130–6510) IU/l	>unl	36	57.1
		≤unl	27	42.9
Hb	11.8 (5.0–15.8) g/dl	Anemia	34	54.0
		No-anemia	29	46.0
β2M	2.08 (1.21–4.44) mg/dl	>unl	30	54.5
		≤unl	25	45.5
sIL-2R	984 (296–7840) IU/ml	>1000 IU/ml	25	48.1
		≤1000 IU/ml	27	51.9

CRP, C-reactive protein; LDH, lactate dehydrogenase; Hb, hemoglobin; β2M, β2-microglobulin; sIL-2R, soluble interleukin-2 receptor; unl, upper normal limit.

Twenty-nine patients were treated with salvage treatment combined with rituximab. Ten patients received autologous hematopoietic stem cell transplantation after salvage therapy.

Response and Survival

Among 50 patients treated with ICE, 12 patients achieved complete remission, 14 patients achieved partial response and, in 18, their disease progressed. The complete remission rate and overall response rate (partial response plus complete remission rate) were 26.7 and 57.8%, respectively. Four patients could not continue ICE because of severe liver damage. Among 13 patients treated with DHAP, 4 patients achieved complete remission, 1 patient achieved partial response and 7 had progressive disease. The complete remission rate and overall response rate (partial response plus complete remission rate) were 33.3 and 41.7%, respectively. One patient could not continue DHAP because of cytomegalovirus infection. After a median follow-up time of 12.3 months (range, 1.3–66.2 months), median OS in all patients was 15.6 months (95% CI 11.6–19.6), and the OS was significantly worse in the DHAP arm than in the ICE arm (5.8 versus 17.6 months, respectively; $P = 0.024$).

Prognostic Factors

The analysis of prognostic factors influencing OS is shown in Table 3. The OS was significantly worse in patients with the following prognostic factors: serum LDH level over the upper normal limit ($P = 0.004$) and hemoglobin under the lower normal limit ($P = 0.001$) as calculated by univariate analysis. The serum CRP levels over the upper normal limit tended to predict poor survival ($P = 0.059$). Moreover, in multivariate analysis, the OS was significantly worse in patients with the following parameters: elevated LDH level (hazard ratio (HR) 3.948. 95% CI 1.385–11.252; $P = 0.010$), anemia (HR 3.925. 95% CI 1.292–11.924; $P = 0.016$) and elevated CRP level (HR 3.757. 95% CI 1.264–11.163; $P = 0.017$).

Table 3. The univariate and multivariate analyses of laboratory parameters that influence the overall survival.

Factors	Cut-off	Univariate	Multivariate	Hazard ratio
CRP	>unl	0.059	0.017	3.757 (1.264–11.163)
LDH	>unl	0.004	0.010	3.948 (1.385–11.252)
Anemia	Positive	0.001	0.016	3.925 (1.292–11.924)
β2M	>unl	0.550	0.325	1.615 (.621–4.200)
sIL-2R	>1000 IU/ml	0.085	0.298	0.580 (.207–1.619)

LAC Index

When the outcome was plotted according to the numbers of elevated CRP level, elevated LDH level and anemia before salvage treatment, three risk groups emerged. Patients with zero or one prognostic factor had the best outcome, and patients with two and three prognostic factors had the poorest outcome. We defined them as low risk and high risk, respectively. We developed an index (elevated LDH, anemia and elevated CRP: LAC index), which represented the numbers of elevated CRP level, elevated LDH level and anemia before salvage therapy, to evaluate risk factors for OS after salvage treatment started. According to the LAC index, among the patients with all lymphoma, the numbers of high- and low-risk patients are 24 and 39, respectively. On the other hand, among the patients with diffuse large B-cell lymphoma, the numbers of high- and low-risk patients are 16 and 25, respectively.

The median OS of high- and low-risk patients was 5.8 months (95% CI 3.3–8.3) and 60.1 months (95% CI 0.0–125.0), respectively. There was a significant difference among high- and low-risk patients in all patients (log-rank test; $P < 0.001$, Fig. 1). Moreover, among the patients with 39 diffuse large B-cell lymphoma patients, excluding two transformed large B-cell lymphoma, the median OS of high- and low-risk patients was 9.5 months (95% CI 1.3–17.7) and 60.1 months (95% CI 0.0–121.0), respectively. There was a significant difference among high- and low-risk patients for patients with diffuse large B-cell lymphoma (log-rank test; $P = 0.014$, Fig. 2).

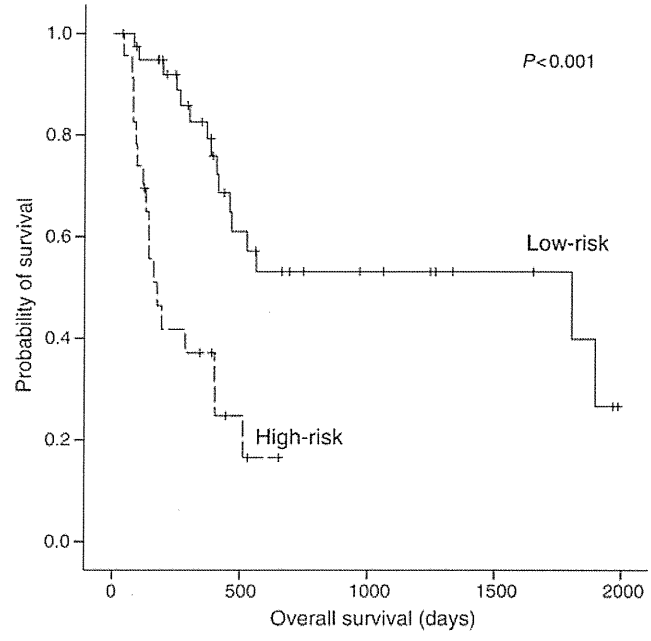

Figure 1. The LAC index among all patients. The median overall survival of high- and low-risk patients were 5.8 months (95% CI 3.3–8.3) and 60.1 months (95% CI 0.0–125.0), respectively. There was a significant difference among high- and low-risk patients in all patients (log-rank test; $P < 0.001$).