Prognostic biomarkers in patients with localized natural killer/T-cell lymphoma treated with concurrent chemoradiotherapy

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Concurrent chemoradiotherapy has become one of the standard management approaches for newly diagnosed localized nasal natural killer (NK)/T-cell lymphoma (NKTCL). Few data are available on the prognostic biomarkers of NKTCL among patients treated with concurrent chemoradiotherapy. To evaluate the prognostic significance of immunophenotypic biomarkers for patients treated with concurrent chemoradiotherapy, latent membrane protein 1 (LMP1), cutaneous lymphocyte antigen (CLA) and cell origin were examined in samples from 32 patients who were enrolled in the Japan Clinical Oncology Group 0211 trial and treated with concurrent chemoradiotherapy. LMP1 and CLA were positive in 66% (19/29) and 29% (9/31) of the cases examined, respectively. The median follow-up duration was 68 months (range, 61–94). The patients with LMP1-positive tumors showed a better overall survival (OS) than the patients with LMP1-negative tumors (hazard ratio, 0.240; 95% confidence interval [CI], 0.057–1.013; 80% CI, 0.093–0.615; $P = 0.035$). All five patients with LMP1-negative tumors who experienced disease progression died of lymphoma, and both patients with local failure had LMP1-negative tumors. There was no significant difference in OS according to CLA expression. A total of 27 (84%) cases were of NK-cell origin, two were of $\alpha\beta$ T-cell origin and three were of $\gamma\delta$ T-cell origin. In contrast to those with tumors of NK-cell origin, all five patients with NKTCL of T-cell origin were alive without relapse at the last follow up. Our results indicate that LMP1 expression is a favorable prognostic marker and suggest that a T-cell origin of the tumor may be a favorable prognostic marker for patients with localized NKTCL treated with concurrent chemoradiotherapy.

Extranodal natural killer (NK)/T-cell lymphoma (NKTCL), nasal type, is a predominantly extranodal lymphoma associated with Epstein–Barr virus (EBV).[1,2] The tumor cells in most cases of NKTCL show an NK-cell phenotype,[1,2] while some cases show a T-cell phenotype, including $\gamma\delta$ T-cell and $\alpha\beta$ T-cell types.[3,4]

Tumor cells of NKTCL express P-glycoprotein, resulting in tumor multidrug resistance.[5–7] The outcomes after treatment with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) or with CHOP-like chemotherapy for localized nasal NKTCL are unsatisfactory.[8–10] Based on the results of clinical trials published in 2009,[11,12] concurrent chemoradio-

therapy has been recognized as one of the standard management approaches for newly diagnosed localized NKTCL.[13–15] In Japan, the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG) conducted a phase I/II study (JCOG0211) of radiotherapy (RT) and dexamethasone, etoposide, ifosfamide and carboplatin (DeVIC) (RT-DeVIC) for newly diagnosed localized nasal NKTCL.[11,16] In patients who were treated with the recommended dose (RT-2/3DeVIC), the 5-year overall survival (OS) and the 5-year progression-free survival (PFS) were 70 and 63%, respectively.[16] Subgroup analysis further revealed that both the international prognostic index[17] and the NK/T-cell lymphoma prognostic

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index[18] were not valid for the patient cohort of JCOG0211,[11] and similar results of a subgroup analysis were obtained in a phase II study of concurrent chemoradiotherapy in Korea.[12]

Latent membrane protein 1 (LMP1),[19–22] cutaneous lymphocyte antigen (CLA),[23,24] NK-cell origin[4,25–27] and EBV-encoded RNA (EBER) in the pretreatment bone marrow (BM), as detected by *in situ* hybridization,[28] have all been reported as prognostic biomarkers in patients with NKTCL who have been treated with conventional therapies. However, the prognostic significance of these biomarkers remains unclear, as most patients with NKTCL in previous studies were treated with heterogeneous treatment modalities. Because concurrent chemoradiotherapy is a new treatment modality for lymphoma, few data are available on the prognostic biomarkers of NKTCL among patients treated with concurrent chemoradiotherapy.

To evaluate the prognostic significance of immunophenotypic biomarkers among patients treated with concurrent chemoradiotherapy, we conducted an ancillary clinicopathologic study of the JCOG0211 trial.

Materials and Methods

Patients, treatment and tissue samples. The subjects in this study included 33 patients who were enrolled in the JCOG0211 trial. The design of the JCOG0211 trial has previously been described in detail.[11] Briefly, patients were eligible for the study if they were 20 to 69 years old and had previously untreated extranodal NKTCL, nasal type.[1] Patients were also required to have stage IE or contiguous stage IIE disease with cervical lymph node involvement and at least one measurable lymphomatous lesion in the nasal cavity and/or its adjacent sites. Patients received RT-DeVIC therapy consisting of RT of 50 Gy and three cycles of DeVIC chemotherapy. A two-thirds dose of DeVIC was selected for 27 patients who were evaluated in the phase II portion of JCOG0211. A full-dose of DeVIC was selected for six patients in the phase I portion. Among 33 cases, four cases had been included in a previous single-center study analyzing LMP1 expression in tumor cells of NKTCL.[21]

Sections of formalin-fixed, paraffin-embedded tissues of pretreatment lymphoma and BM samples were collected from the patients. The histological diagnoses of all patients were
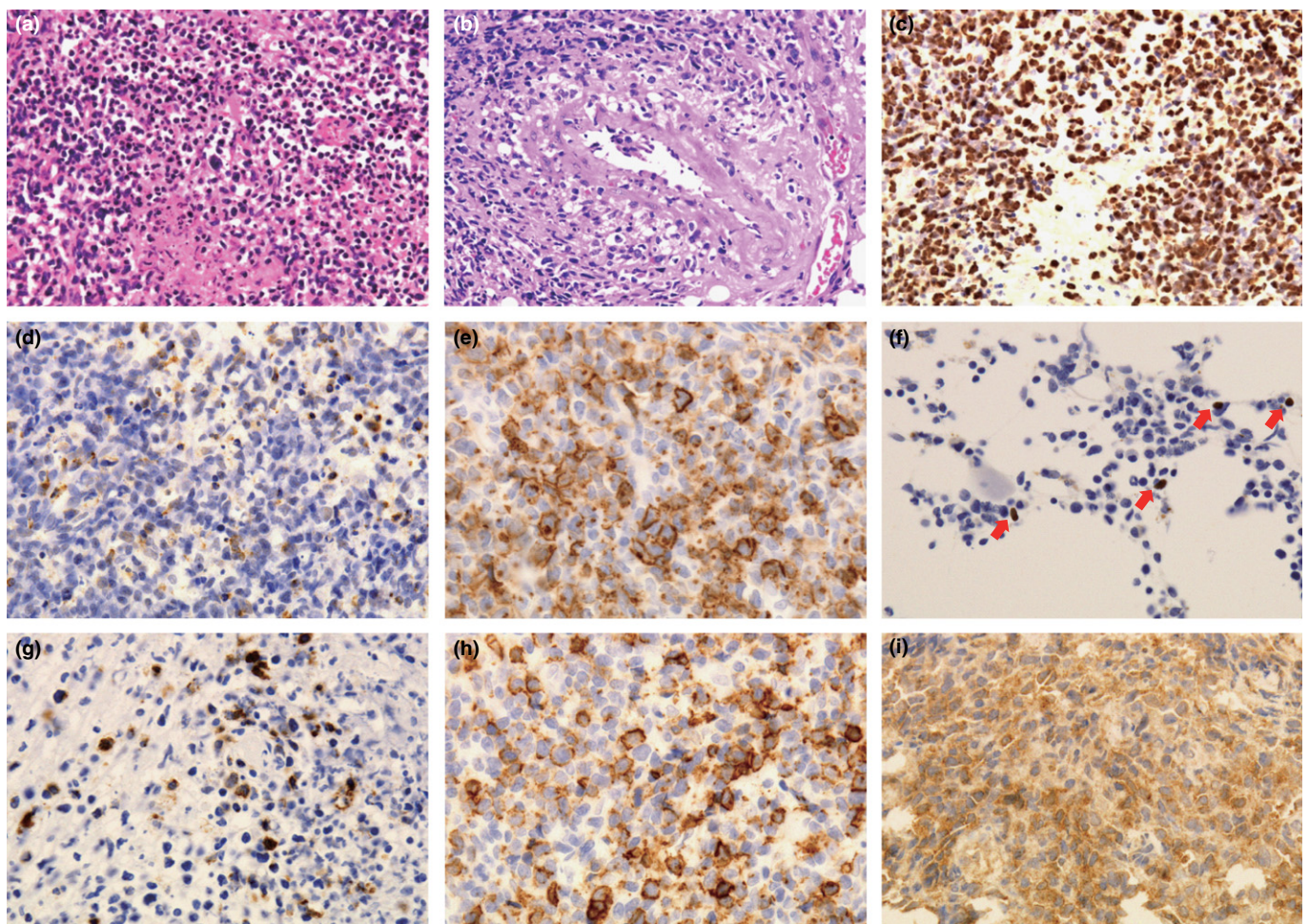

Fig. 1. (a) H&E staining in NK/T-cell lymphoma (NKTCL) of $\gamma\delta$ T-cell origin (57-year-old male patient). Medium to large sized tumor cells with necrosis are seen. (b) H&E staining in NKTCL of $\gamma\delta$ T-cell origin (34-year-old male patient). Tumor cells have an angiocentric angiodestructive quality. (c) *In situ* hybridization study for NKTCL of $\gamma\delta$ T-cell origin (57-year-old male patient). Tumor cells are positive for Epstein–Barr virus encoded RNA (EBER). (d) Neoplastic tissue from the nasal cavity of a 39-year-old female patient with NKTCL of NK-cell origin. The neoplastic cells are positive for latent membrane protein 1 (LMP1). (e) Neoplastic tissue from the oral cavity of a 57-year-old male patient with NKTCL of NK-cell origin. The neoplastic cells are positive for cutaneous lymphocyte antigen (CLA). (f) Bone marrow tissue from a 34-year-old male patient with NKTCL of $\gamma\delta$ T-cell origin. There are a small number of EBER-positive cells (red arrows). (g–i) Neoplastic tissue from the nasal cavity of a 55-year-old male patient with NKTCL of $\gamma\delta$ T-cell origin. The neoplastic cells are positive for LMP1 (g), CLA (h) and T-cell receptor γ (i).

confirmed as extranodal NKTCL, nasal type by the Central Pathology Review Board.[11] All immunohistopathological examinations for the current ancillary study were performed at the Central Pathology Office of the ancillary study (Okayama University Hospital, Okayama, Japan).

The current study was approved by the JCOG Protocol Review Committee and the institutional review board at each study site. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. All data on baseline features, treatment details, response and follow up were retrieved from the original JCOG0211 dataset.

Immunohistochemical analysis. Immunohistochemical staining was performed on sections of formalin-fixed, paraffin-embedded tissues of pretreatment lymphoma samples with heat-induced or trypsin-induced epitope retrieval using an avidin–biotin complex method and an automated immunostainer (Bond-max, Leica Biosystems, Melbourne, Vic., Australia), as previously described.[29] The following primary antibodies were used to assess these samples: LMP1 (CS1-4, 1:50, Novocastra, Newcastle-upon-Tyne, UK), T-cell receptor (TCR) β (βF1; TCR1151, 8A3, 1:50, Thermo Scientific, Waltham, MA, USA), TCRγ chain constant region (CγM1; TCR1153, γ3.20, 1:80, Thermo Scientific) and CLA (HECA452, 1:10) as previously described.[23,29]

For the LMP1 antigen, samples were determined to be positive when the lymphoma cells were positive according to the methods described by Kanemitsu *et al.* [21]. For βF1, CγM1 and CLA expression, samples in which 30% or more of the cells expressed the antigen were scored as positive, as previously described.[23,29] A preliminary evaluation for the study showed that staining for TCRγ and TCRδ was concordant in all cases.[29] Cases were considered to be of NK-cell origin if both TCRβ and TCRγ expression was not observed. Cases with positive staining for one or both of the antibodies (TCRβ and TCRγ) were determined to be of T-cell origin.[29]

In situ hybridization. Pretreatment BM specimens from patients were examined for EBER. *In situ* hybridization with EBER-1 probes (INFORM EBER, Leica Biosystems, Melbourne, Victoria, Australia) was performed to detect EBV.[29]

Statistical analysis. Survival estimates were calculated using the Kaplan–Meier method. Hazard ratios (HR) and 80 and 95% confidence intervals (CI) were estimated using a Cox regression. All of the analyses were performed using IBM SPSS Statistics 20.0 (IBM Japan, Tokyo, Japan).

Results

Expression of biomarkers. Pathological samples from 32 out of 33 patients were available for this study. Tissue samples from the remaining patient were exhausted during the Central Pathology Review and, therefore, were no longer available for the current study. This patient was a 33-year-old woman with

Table 1. Clinical features according to each biomarker expression

	All Patients n = 32 (%)	LMP1		CLA		Cell Origin	
		Positive n = 19	Negative n = 10	Positive n = 9	Negative n = 22	NK-cell type n = 27	T-cell type n = 5
Age at Diagnosis, n (%)							
≤60	25 (78)	15 (79)	8 (80)	8 (89)	16 (73)	21 (78)	4 (80)
>60	7 (22)	4 (21)	2 (20)	1 (11)	6 (27)	6 (22)	1 (20)
Sex, n (%)							
Male	19 (59)	9 (47)	3 (30)	9 (100)	10 (45)	12 (44)	1 (20)
Female	13 (41)	10 (53)	7 (70)	0 (0)	12 (55)	15 (56)	4 (80)
PS, n (%)							
0 or 1	30 (94)	17 (89)	10 (100)	9 (100)	20 (91)	25 (93)	5 (100)
>1	2 (6)	2 (11)	0 (0)	0 (0)	2 (9)	2 (7)	0 (0)
Serum LDH Level, n (%)							
≤Normal	25 (78)	15 (79)	7 (70)	7 (78)	17 (77)	20 (74)	5 (100)
>Normal	7 (22)	4 (21)	3 (30)	2 (22)	5 (23)	7 (26)	0 (0)
B Symptoms, n (%)							
Absent	20 (63)	13 (68)	5 (50)	4 (44)	15 (68)	16 (59)	4 (80)
Present	12 (37)	6 (32)	5 (50)	5 (56)	7 (32)	11 (41)	1 (20)
Stage, n (%)							
IE	22 (69)	12 (63)	8 (80)	5 (56)	16 (73)	20 (74)	2 (40)
IIE	10 (31)	7 (37)	2 (20)	4 (44)	6 (27)	7 (26)	3 (60)
Skin Involvement, n (%)							
Absent	18 (56)	14 (74)	3 (30)	4 (44)	14 (64)	13 (48)	5 (100)
Present	14 (44)	5 (26)	7 (70)	5 (56)	8 (36)	14 (52)	0 (0)
IPI, n (%)							
0	19 (59)	11 (58)	6 (60)	6 (67)	12 (55)	15 (56)	4 (80)
1	10 (31)	6 (32)	3 (30)	3 (33)	7 (32)	9 (33)	1 (20)
2	3 (9)	2 (11)	1 (10)	0 (0)	3 (14)	3 (11)	0 (0)
NK-PI, n (%)							
0	11 (34)	7 (37)	3 (30)	1 (11)	9 (41)	9 (33)	2 (40)
1	9 (28)	4 (21)	4 (40)	5 (56)	4 (18)	8 (30)	1 (20)
2	9 (28)	7 (37)	1 (10)	2 (22)	7 (32)	7 (26)	2 (40)
3	3 (9)	1 (5)	2 (20)	1 (11)	2 (9)	3 (11)	0 (0)

CLA, cutaneous lymphocyte antigen; IPI, international prognostic index; LDH, lactate dehydrogenase; LMP1, latent membrane protein 1; NK-PI, NK/T-cell lymphoma prognostic index; PS, performance status.

stage IIE NKTCL who obtained a complete response (CR) by RT-(full-dose) DeVIC and was alive at the last follow-up examination (86 months). Pretreatment BM samples were obtained from 29 of the 32 patients.

The immunohistochemical features of these samples are shown in Figure 1. LMP1 and CLA were positive in 66% (19 /29) and 29% (9/31) of the cases examined, respectively. Among the 32 cases that were examined to determine cell lineage, 27 (84%) cases were of NK-cell origin. Two (6%) cases were of αβ T-cell origin on the basis of TCRβ immunoreactivity but not TCRγ, and 3 (9%) cases were of γδ T-cell origin due to the presence of TCRγ immunoreactivity but not TCRβ immunoreactivity. Pretreatment BM samples were positive for EBER in 2 (7%) out of 29 cases examined. One of the two EBER-positive cases had two positive cells in a total field of view. Another case had three to five positive cells/high-power field. Positive cells were small to medium sized cells and were diffusely distributed in the latter case.

The Central Pathology Review of JCOG0211 confirmed that tumor cells in 29 out of the 32 cases were positive for EBER. Tissue samples of the remaining three cases were not evaluable for EBER. The current study revealed LMP1 expression in tumor cells of all the remaining three cases, indicating that all 32 cases were associated with EBV.

In the 29 patients whose samples were available for analysis of LMP1 and CLA expression, tumors from four patients were positive for both antigens, tumors from 15 patients were positive for LMP1 only, tumors from four patients were positive for CLA only, and tumors from six patients were negative for both antigens. In the three patients with NKTCL of γδ T-cell origin, two samples contained cells that were positive for both LMP1 and CLA, and one sample contained cells that were negative for both antigens. Of the two cases of αβ T-cell origin, one showed an LMP1-positive and CLA-negative immunophenotype, while another case was positive for both LMP1 and CLA. In two patients whose BM samples were positive for EBER, one had NKTCL of NK-cell origin that was negative for LMP1 and CLA, and the other had NKTCL of γδ T-cell origin that was positive for both LMP1 and CLA.

Clinical features according to the expression of each biomarker. The baseline clinical characteristics according to the expression of each biomarker are shown in Table 1. All CLA-positive tumors in the study were from male patients. Skin involvement prior to treatment was frequently observed in the patients with LMP1-negative tumors (odds ratio [OR], 6.533 [95% CI, 1.200–35.573]). All five patients with NKTCL of T-cell origin showed no skin involvement at baseline (OR, 0.722 [95% CI, 0.542–0.962]). No significant correlation was observed between the induction of CR and the expression of each biomarker (data not shown).

Survival analysis. The median follow-up duration was 68 months (range, 61–94). The OS was better in the LMP1-positive group than in the LMP1-negative group (HR, 0.240; 95% CI, 0.057–1.013; 80% CI, 0.093–0.615; $P = 0.035$, Fig. 2). The OS at 5 years was 84% in the LMP1-positive group and 50% in the LMP1-negative group. The PFS at 5 years was 74% in the LMP1-positive group and 50% in the LMP1-negative group. The HR of PFS among LMP1-positive tumors was 0.421 (95% CI, 0.121–1.463; 80% CI, 0.187–0.950). There were no significant differences in the OS and the PFS between the CLA-positive and CLA-negative groups (5-year OS, 56 vs 77%; 5-year PFS, 56 vs 68%, Fig. 2). All
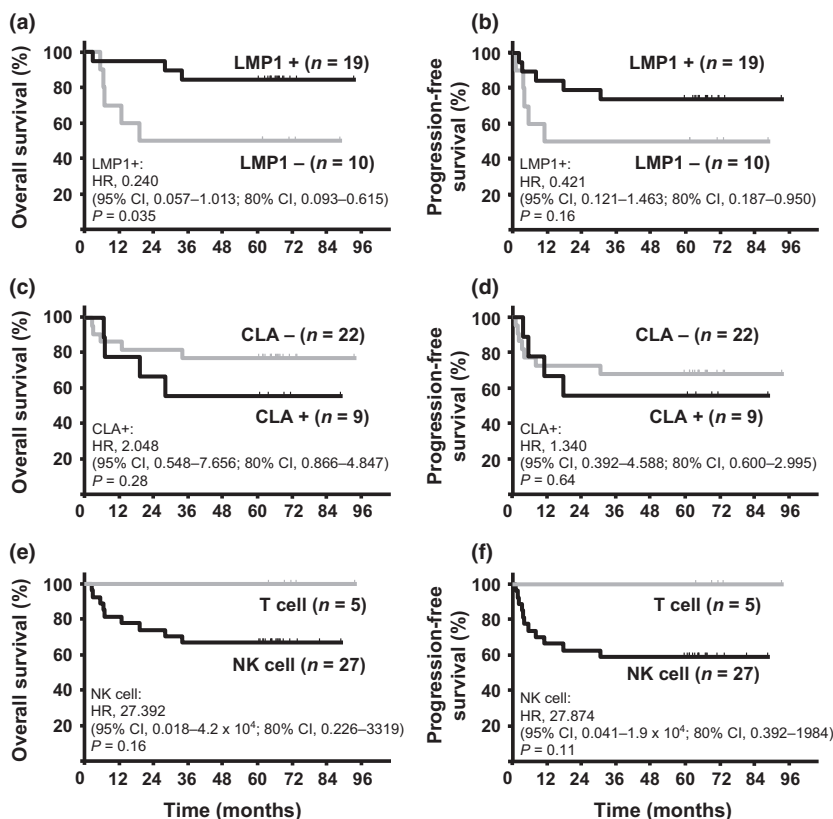

Fig. 2. (a,c,e) Overall survival curves of patients with NK/T-cell lymphoma (NKTCL) by latent membrane protein 1 (LMP1) expression (a), cutaneous lymphocyte antigen (CLA) expression (c) and cell-of-origin (e). (b,d,f) Progression-free survival curves of patients with NKTCL by LMP1 expression (b), CLA expression (d) and cell-of-origin (f).

Table 2. Characteristics and biomarker expression of the 11 patients who experienced disease progression or recurrence during follow-up

Age/Sex	Stage	LMP1	CLA	Overall response	Site(s) of recurrence	Salvage therapy	OS, mo	PFS, mo	Outcome
57/F	IIEA	+	−	PD	Liver, spleen, BM	None	3	2	DOD
21/M	IEA	+	−	PD	LN	Chemotherapy for ALL, CBT	69	3	AND
57/F	IIEA	+	−	CR	LN, stomach	L-asp/VCR/DMS, Allo PBSCT	68	8	AND
57/M	IIEB	+	−	CR	BM, PB	CMED, L-asp/VCR/DMS, EPOCH	34	31	DOD
38/M	IIEB	+	+	CR	LN, liver, spleen, ascites	mPSL, ETP, FCM	29	18	DOD
58/M	IEB	−	+	CR	Skin	ESHAP, RT, CHOP	19	11	DOD
63/F	IEA	−	−	PD	CNS, nasal cavity†, parotid gland, PB, subcutaneous tissue	MTX/AraC it, IVAC, HD-MTX, DeVIC, CHOP	13	1	DOD
57/M	IIEA	−	+	CR	LN, skin, BM	L-asp/VCR/DMS	7	4	DOD
58/F	IEA	−	−	PR	Skin, kidney, LN	ESHAP	6	4	DOD
60/M	IEB	−	+	SD	Eye, nasal cavity†, skin	None	7	6	DOD
45/M	IIEB	ND	−	PD	Gallbladder, BM	None	3	2	DOD

ALL, acute lymphoblastic leukemia; Allo PBSCT, allogeneic peripheral blood stem cell transplantation; AND, alive with no evidence of disease; AraC, cytarabine; BM, bone marrow; CBT, cord blood transplantation; CLA, cutaneous lymphocyte antigen; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; CMED, cyclophosphamide, methotrexate, etoposide, and dexamethasone; CNS, central nervous system; CR, complete response; DeVIC, dexamethasone, etoposide, ifosfamide, and carboplatin; DOD, died of disease; EPOCH, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; ESHAP, etoposide, methylprednisolone, cytarabine, and cisplatin; ETP, etoposide; FCM, fludarabine, cyclophosphamide, and mitoxantrone; HD, high dose; it, intrathecal; IVAC, ifosfamide, etoposide, and cytarabine; L-asp/VCR/DMS, L-asparaginase, vincristine, and dexamethasone; LMP1, latent membrane protein 1; LN, lymph node, mPSL, methylprednisolone; MTX, methotrexate; ND, not done; OS, overall survival; PB, peripheral blood; PD, progressive disease; PFS, progression-free survival; PR, partial response; RT, radiotherapy; SD, stable disease. All 11 cases were of NK-cell origin. †Local failure.

five patients with NKTCL of T-cell origin achieved a CR by RT-DeVIC and were alive, without relapse, at the time of the last follow up (Fig. 2). The 5-year OS and PFS in the 27 patients with NKTCL of NK-cell origin were 67 and 59%, respectively. The two patients whose pretreatment BM samples were positive for EBER survived for more than 5 years without disease progression.

Characteristics and biomarker expression in patients who experienced disease progression or recurrence. During the follow-up period, 11 of the 33 patients who were enrolled in JCOG0211 experienced disease progression or recurrence. Detailed clinical information and biomarker expression for these cases are presented in Table 2. Among the 5 LMP1-positive patients, two patients achieved a second CR using L-asparaginase-containing chemotherapy followed by allogeneic hematopoietic stem cell transplantation and attained long-term survival. In contrast, all five patients whose tumors were LMP1-negative and who experienced disease progression died within 19 months of the initial registration. In JCOG0211, only two patients experienced local failure,[16] and the present study revealed that both of these cases were LMP1-negative (Table 2).

Discussion

The current study showed that LMP1 expression in tumor cells was associated with OS in patients with newly diagnosed localized NKTCL who were treated uniformly with concurrent chemoradiotherapy. Of note, all five patients with NKTCL of the T-cell type, including three patients with NKTCL of the γδ T-cell type, survived more than 5 years without recurrence.

Although NKTCL is associated with a type II EBV latency program,[30] the expression levels of LMP1 in NKTCL is variable at the single-cell level.[20,21,30] In addition, there have been conflicting results in previous studies analyzing the prognostic significance of LMP1 expression in NKTCL. A single-center study of 58 patients with NKTCL (advanced disease, n = 12) in China reported that patients with LMP1-positive

tumors showed significantly worse survival.[19] Another study from China, which included 16 patients with NKTCL (advanced disease, n = 2), reported that patients whose tumors exhibited high LMP1 expression (81–100% of tumor cells) showed significantly shorter survival.[20] In contrast, one single-center study from Japan, which included 30 patients with NKTCL (advanced disease, n = 7), showed a favorable outcome for patients whose tumors were LMP1-positive compared to patients whose tumors were LMP1-negative.[21] Possible explanations for the incongruence in these reported results include the heterogeneous therapeutic approaches used, the differences in the incidence of advanced stage, and the use of different criteria for LMP1 positivity.

In the present study, the lack of LMP1 expression was associated with a short OS in patients with localized NKTCL who were treated with RT-DeVIC. Skin involvement was frequently observed in LMP1-negative cases. All five patients with LMP1-negative tumors who experienced disease progression died, and the two patients who experienced local failure in JCOG0211 had tumors that were LMP1-negative. Together, these results indicate that LMP1-negative NKTCL has an aggressive nature. The lack of detectable LMP1 in aggressive NK-cell leukemia[31] may also support this assertion. In LMP1-negative tumors, some researchers speculate that additional cellular genetic aberrations may be driving a more malignant tumor phenotype that no longer requires expression of the LMP1 oncogene.[32]

Differences in prognosis between NKTCL patients with the NK-cell type and the T-cell type have been the subject of previous research. Early studies revealed that patients with CD56-positive NKTCL show significantly worse survival than those with CD56-negative NKTCL.[25,26] A Chinese group reported that there was no difference in prognosis between these two groups.[27] However, patients in these studies were treated with various treatment modalities and different chemotherapeutic regimens. Recent immunohistochemical analyses using monoclonal antibodies against the TCRβ, γ and δ subunits have shown that some CD56-positive NKTCL cases are actually γδ

T-cell lymphoma and that NKTCL of T-cell origin exhibits a trend for better OS than NKTCL of NK-cell origin.[4] In the current study, patients were uniformly treated with RT-DeVIC. Moreover, the origins of the lymphoma cells were determined immunohistochemically with monoclonal antibodies against the TCRβ and γ subunits, which constitutes a more specific method of identifying cases of the T-cell type than the previous approaches. Our current study highlighted the favorable prognosis of patients with NKTCL of T-cell origin, which suggests that a more effective therapeutic strategy is needed for NKTCL of NK-cell origin.

CLA is a skin-homing receptor that functions in the adhesion of cells to the vascular endothelium.[33] CLA-positive lymphocytes that migrate to the skin consist mostly of T cells, but NK cells are also present in this population.[34] Patients with NKTCL often develop skin involvement during the clinical course of the disease.[1] In the present study, 5 (56%) out of nine patients with CLA-positive tumors also had skin lesions. Yoshino et al.[23] examined CLA expression in 52 patients with NKTCL, including 14 patients with advanced disease, and found that CLA expression in NKTCL was associated with skin involvement and a poor prognosis.[23] A retrospective analysis with a small number of patients showed similar results in terms of survival.[24] In the present study, CLA expression was not associated with a poor prognosis. One possible reason is that only patients with NKTCL localized within the nasal cavity or the nasopharyngeal region were included in our study. It is also possible that the prognosis of patients with CLA-positive tumors may have been improved by RT-DeVIC.

In the current study, we examined the prognostic significance of the presence of EBER in pretreatment BM samples from patients, partly because testing for BM EBER was not routinely performed in Japan during the study period (2003–2006) of the JCOG0211 trial. Two patients in our study with EBER-positive pretreatment BM survived more than 5 years without disease progression, which suggests that EBER-positive BM did not affect the prognosis of our JCOG0211 patient cohort.

The current study demonstrated, for the first time, the favorable impact of LMP1 expression on the OS of patients with newly diagnosed localized nasal NKTCL in a cohort of patients uniformly treated with concurrent chemoradiotherapy. The limitations of this study include the relatively small number of patients included, the lack of available data on the EBV DNA load in the peripheral blood, which has been reported as a useful biomarker of NKTCL,[35,36] and the possibility of intermingled TCR silent cases in our NKTCL cases of NK-cell origin. Although our evaluations were confined to a relatively small number of patients, these patients were derived from a uniform cohort and were prospectively treated with the same therapeutic strategy consisting of RT-DeVIC. The prognostic values of LMP1 expression and the T-cell origin warrant further evaluation in future studies with a larger number of patients with NKTCL who are treated with concurrent chemoradiotherapy.

Disclosure Statement

K. Oshimi is an employee of Eisai. The remaining authors have no conflict of interest to declare.

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