patients in the era of highly active antiretroviral therapy. Blood 99:4250–4251.
12. Wolf, T., H. R. Brodt, S. Fichtlscherer, K. Mantzsch, D. Hoelzer, E. B. Helm, et al. 2005. Changing incidence and prognostic factors of survival in AIDS-related non-Hodgkin's lymphoma in the era of highly active antiretroviral therapy (HAART). Leuk. Lymphoma 46: 207–215.
13. Glaser, S. L., C. A. Clarke, M. L. Gulley, F. E. Craig, J. A. DiGiuseppe, R. F. Dorfman, et al. 2003. Population-based patterns of human immunodeficiency virus-related Hodgkin lymphoma in the greater San Francisco Bay Area, 1988–1998. Cancer 98:300–309.
14. Little, R. F., S. Pittaluga, N. Grant, S. M. Steinberg, M. F. Kavlick, H. Mitsuya, et al. 2003. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. Blood 101:4653–4659.
15. Ledergerber, B., A. Telenti, and M. Egger. 1999. Risk of HIV related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: prospective cohort study. Swiss HIV cohort study. BMJ 319:23–24.
16. Besson, C., A. Goubar, J. Gabarre, W. Rozenbaum, G. Pialoux, F. P. Chatelet, et al. 2001. Changes in AIDS-related lymphoma since the era of highly active antiretroviral therapy. Blood 98:2339–2344.
17. Hishima, T., N. Oyaizu, T. Fujii, N. Tachikawa, A. Ajisawa, M. Negishi, et al. 2006. Decrease in Epstein-Barr virus-positive AIDS-related lymphoma in the era of highly active antiretroviral therapy. Microbes Infect. 8:1301–1307.
18. Biggar, R. J., E. S. Jaffe, J. J. Goedert, A. Chaturvedi, R. Pfeiffer, and E. A. Engels. 2006. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. Blood 108:3786–3791.
19. Sukpanichnant, S. 2004. Analysis of 1983 cases of malignant lymphoma in Thailand according to the World Health Organization classification. Hum. Pathol. 35: 224–230.
20. Sriplung, H., and D. M. Parkin. 2004. Trends in the incidence of acquired immunodeficiency syndrome-related malignancies in Thailand. Cancer 101:2660–2666.
21. Swerdlow, S. H., E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, et al., eds. 2008. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France.
22. M. Raphael, J. Said, B. Borisch, E. Cesarman, and N. L. Harris. 2008. Lymphomas associated with HIV infection. Pp. 340–342 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds.WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France.
23. Stein, H., R. A. Warnke, W. C. Chan, E. S. Jaffe, J. K. C. Chan, K. C. Gatter, et al. 2008. Diffuse large B-cell lymphoma, not otherwise specified. Pp. 233–237 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France
24. Leoncini, L., M. Raphael, H. Stein, N. L. Harris, E. S. Jaffe, and P. M. Kluin. 2008. Burkitt lymphoma. Pp. 262–264 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France.
25. Kluin, P. M., N. L. Harris, H. Stein, L. Leoncini, M. Raphael, E. Campo, et al. 2008. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. Pp. 265–266 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France
26. Stein, H., N. L. Harris, and E. Campo. 2008. Plasmablastic lymphoma. Pp. 256–257 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France
27. Isaacson, P. G., E. Campo, and N. L. Harris. 2008. Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease. Pp. 258–259 *in* S. H. Swerdlow, E. Campo, N. L. Harris, E. S. Jaffe, S. A. Pileri, H. Stein, J. Thiele, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 4th ed. International Agency for Research on Cancer (IARC), Lyon, France
28. Hoffmann, C., M. Tiemann, C. Schrader, D. Janssen, E. Wolf, M. Vierbuchen, et al. 2005. AIDS-related B-cell lymphoma (ARL): correlation of prognosis with differentiation profiles assessed by immunophenotyping. Blood 106:1762–1769.
29. Howe, J. G., and J. A. Steitz. 1986. Localization of Epstein-Barr virus-encoded small RNAs by in situ hybridization. Proc. Natl Acad. Sci. USA 83:9006–9010.
30. Katano, H., Y. Sato, T. Kurata, S. Mori, and T. Sata. 1999. High expression of HHV-8-encoded ORF73 protein in spindle-shaped cells of Kaposi's sarcoma. Am. J. Pathol. 155:47–52.
31. Foot, N. J., R. G. Dunn, H. Geoghegan, B. S. Wilkins, and M. J. Neat. 2011. Fluorescence in situ hybridisation analysis of formalin-fixed paraffin-embedded tissue

sections in the diagnostic work-up of non-Burkitt high grade B-cell non-Hodgkin's lymphoma: a single centre's experience. J. Clin. Pathol. 64:802–808.
32. Hans, C. P., D. D. Weisenburger, T. C. Greiner, R. D. Gascoyne, J. Delabie, G. Ott, et al. 2004. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275–282.
33. Chadburn, A., A. Chiu, J. Y. Lee, X. Chen, E. Hyjek, A. H. Banham, et al. 2009. Immunophenotypic analysis of AIDS-related diffuse large B-cell lymphoma and clinical implications in patients from AIDS Malignancies Consortium clinical trials 010 and 034. J. Clin. Oncol. 27:5039–5048.
34. Yamaguchi, M., M. Seto, M. Okamoto, R. Ichinohasama, N. Nakamura, T. Yoshino, et al. 2002. De novo $CD5^+$ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. Blood 99:815–821.
35. Diebold, J., E. S. Jaffe, M. Raphael, and R. A. Warnke. 2001. Burkitt lymphoma. Pp. 181–184 *in* E. S. Jaffe, N. L. Harris, H. Stein, and J. W. Vardiman, eds. WHO classification of tumours of haematopoetic and lymphoid tissues. 3rd ed. IARC Press, Lyon, France.
36. Dave, S. S., K. Fu, G. W. Wright, L. T. Lam, P. Kluin, E. J. Boerma, et al. 2006. Molecular diagnosis of Burkitt's lymphoma. N. Engl. J. Med. 354:2431–2442.
37. Hummel, M., S. Bentink, H. Berger, W. Klapper, S. Wessendorf, T. F. Barth, et al. 2006. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N. Engl. J. Med. 354:2419–2430.
38. Salaverria, I., and R. Siebert. 2011. The gray zone between Burkitt's lymphoma and diffuse large B-cell lymphoma from a genetics perspective. J. Clin. Oncol. 29:1835–1843.
39. Yotsumoto, M., S. Hagiwara, A. Ajisawa, J. Tanuma, T. Uehira, H. Nagai, et al. 2012. Clinical characteristics of human immunodeficiency virus-associated Hodgkin lymphoma patients in Japan. Int. J. Hematol. 96:247–253.

Appendix

Table A1. Effect of ART on the onset of ARL in the ART era.

Factors	ART (–)	ART (+)	*P*
n	105	52	–
Histology			
DLBCL	44 (41.9%)	21 (40.4%)	0.856 (C)
(non-GC/GC)	(26/9)	(10/8)	0.1665 (C)
BL	33 (31.4%)	18 (34.6%)	0.688 (C)
PBL	13 (12.4%)	3 (5.8%)	0.197 (C)
PEL	5 (4.8%)	1 (1.9%)	0.667 (CY)
HL	1 (1.0%)	6 (11.5%)	**<0.01 (CY)**
LBL-KSHV-MCD	0 (0%)	1 (1.9%)	0.719 (CY)
Other	9 (8.6%)	2 (3.8%)	0.448 (C)
Age, years (mean) [median, range]	45.1 [42.6, 25–76]	49.2 [48.5, 30–75]	**<0.01 (MW)**
Men (%)	97	96	0.880 (CY)
CD4 (mean) [median, range]	121.5 [76, 0–560]	280 [184, 6–2431]	**<0.01 (MW)**
EBV-positive (%)	59	44	0.076 (C)
CNS involvement (%)	20	20	0.981 (C)
LN involvement (%)	49	59	0.275 (C)
BM involvement (%)	30	35	0.556 (C)
1-year survival rate (%)	60	66	0.504 (C)

157 cases in the ART era (1997-) were analyzed. *P* values were calculated using the Chi-square test (C), Chi-square test with Yates correction (CY), and Mann–Whitney test (MW). See Table 1 for abbreviations. *P* values < 0.05 are presented in bold.

Japanese Journal of Clinical Oncology Advance Access published February 20, 2014

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol
doi:10.1093/jjco/hyu012

Original Article

Clinical Outcomes of AIDS-related Burkitt Lymphoma: A Multi-institution in Japan

Yuki Kojima[1], Shotaro Hagiwara[2], Tomoko Uehira[3], Atsushi Ajisawa[4], Akira Kitanaka[5], Junko Tanuma[6], Seiji Okada[7] and Hirokazu Nagai[1,*]

[1]Department of Hematology, National Hospital Organization Nagoya Medical Center, Nagoya, [2]Division of Hematology, Internal Medicine, National Center for Global Health and Medicine, Shinjuku, [3]Department of Infectious Diseases, National Hospital Organization Osaka Medical Center, Osaka, [4]Division of Infectious Disease, Tokyo Metropolitan Center and Infectious Disease Center Komagome Hospital, Tokyo, [5]Faculty of Medicine, Miyazaki University, Miyazaki, [6]AIDS Clinical Center, National Center for Global Health and Medicine, Shinjuku and [7]Center for AIDS Research, Kumamoto University, Kumamoto, Japan

*For reprints and all correspondence: Hirokazu Nagai, Department of Hematology, National Hospital Organization Nagoya Medical Center, 4–1-1 Sannomaru, Naka-ku, Nagoya 460-0001, Japan. E-mail: nagaih@nnh.hosp.go.jp

Received July 29, 2013; accepted January 21, 2014

Objective: Acquired immunodeficiency syndrome-related non-Hodgkin lymphoma is treated similarly to non-acquired immunodeficiency syndrome lymphoma, but it is not clear whether highly intensive regimens are beneficial for acquired immunodeficiency syndrome-related Burkitt lymphoma. We conducted a multicenter retrospective survey to clarify the clinical outcomes of acquired immunodeficiency syndrome-related Burkitt lymphoma in the combined antiretroviral therapy era in Japan.

Methods: We retrospectively analyzed the outcome of 33 patients with acquired immunodeficiency syndrome-related Burkitt lymphoma, who were diagnosed at five regional hospitals for human immunodeficiency virus/acquired immunodeficiency syndrome in Japan between January 2002 and December 2010.

Results: The median follow-up period was 20.0 months (range 0.5–92.7 months). Six (18.2%) patients were treated with cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate, ifosphamide, etoposide and high-dose cytarabine, and 23 (69.7%) patients were treated with hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, high-dose methotrexate and high-dose cytarabine. The overall response rate for all patients was 78.8%, with a complete response rate of 72.7%. The two-year overall survival rate was 68.1%. There was no significant difference in overall survival between chemotherapeutic regimens with rituximab ($n = 20$) and without rituximab ($n = 13$) ($P = 0.49$). The two-year overall survival rate was 66.7% for patients receiving cyclophosphamide, vincristine, doxorubicin, dexamethasone, etoposide, ifosfamide and cytarabine, and was 72.6% for patients receiving cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine ($P = 0.72$). There was one treatment-related death.

Conclusions: Highly intensive chemotherapy would bring a high remission rate and prolonged overall survival for patients with acquired immunodeficiency syndrome-related Burkitt lymphoma.

Key words: Burkitt lymphoma – AIDS-related – chemotherapy – rituximab – survival

INTRODUCTION

Combination antiretroviral therapy (cART) has dramatically reduced the occurrence of opportunistic infections such as acquired immunodeficiency syndrome (AIDS)-defining illnesses (ADIs). Decreases in the incidence of AIDS-related lymphoma (ARL) are not as evident compared with other ADIs; thus, lymphoma is now a more common cause of AIDS-related mortality. Adult Burkitt lymphoma (BL) comprises 1–5% of cases of non-Hodgkin lymphoma (1). However, BL in human immunodeficiency virus (HIV) patients is a typical AIDS-defining cancer (ADC) and accounts for 25–40% of ARLs (2–4). AIDS-related BL (AIDS-BL) occurs even in subjects with higher CD4 + lymphocyte counts (3, 5–10), and is therefore important even in patients who are receiving effective therapy for HIV. AIDS-BL progresses very rapidly and is associated with a poor prognosis. In spite of the widespread use of cART, the survival of patients with AIDS-related diffuse large B-cell lymphoma (DLBCL) and that of patients with AIDS-BL remain poor (11, 12). Recent studies have demonstrated that highly intensive chemotherapeutic regimens, such as cyclophosphamide, vincristine, doxorubicin, dexamethasone, etoposide, ifosfamide and cytarabine (CODOX-M/IVAC) or cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine (hyper-CVAD/MA), have improved the survival of patients with non-HIV BL. Thus, the poor outcomes associated with AIDS-BL in previous reports might have resulted from the use of less intensive treatment strategies, such as cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)-based chemotherapy (13–15). The introduction of cART has resulted in improved general health for patients with HIV; thus, the eligibility of these patients to receive cytotoxic agents has increased to the point where it is nearly similar of that of the non-HIV population, and these patients now would be candidates for highly intensive chemotherapy regimens. The CD20-directed monoclonal antibody, rituximab can improve survival when added to standard chemotherapy in patients with low-grade B-cell lymphomas and DLBCL (16–18). Also the addition of rituximab in non-HIV BL has been examined by randomized clinical trials. However, the impact of rituximab for patients with AIDS-BL patients is unknown. Therefore, we performed a nationwide retrospective survey to elucidate the clinical outcomes and to identify significant prognostic variables for AIDS-BL in the cART era.

PATIENTS AND METHODS

Patients

This retrospective study examined the clinical outcomes of all untreated patients with AIDS-BL who visited one of five regional hospitals for HIV/AIDS in Japan, from January 2002 to December 2010. The pathological diagnosis of each institution was accepted. The pathological diagnosis of BL was based on the 2008 World Health Organization (WHO) classification. AIDS-BL was defined as immunodeficiency-associated BL of the 2008 WHO classification. Examination for *myc* gene alterations was not mandatory.

Chart reviews were performed for all identified patients to obtain the following information: age, sex, performance status (PS) at diagnosis, number of CD4 + cells at diagnosis, prior AIDS, concurrent opportunistic diseases, prior cART, clinical stage, lactate dehydrogenase (LDH), the International Prognostic Index (IPI), disease site, presence of central neurological system (CNS) involvement, presence of bone marrow (BM) involvement, chemotherapy regimen, tumor response and clinical outcomes. This study received approval from the appropriate ethics committees.

Definitions of Terms and Treatment

The PS was evaluated according to the Eastern Cooperative Oncology Group scale (19). Clinical stage was assessed using the Ann Arbor staging system (20). The IPI was assessed according to the International Non-Hodgkin Lymphoma Prognostic Factors project (21). The response was assessed according to the International Workshop Criteria for non-Hodgkin lymphoma (22). Patients were classified according to tumor response: complete response (CR), CR unconfirmed (CRu), partial response (PR), stable disease (SD) or disease progression (PD). The overall response rate (ORR) was calculated as the proportion of patients who achieved a CR, CRu or PR.

The CODOX-M/IVAC regimen consisted of cyclophosphamide, vincristine, doxorubicin and high-dose methotrexate, alternating with etoposide, ifosfamide and high-dose cytarabine (23). The hyper-CVAD/MA regimen consisted of hyperfractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone, alternating with high-dose methotrexate and cytarabine (24). The EPOCH regimen consisted of etoposide, vincristine, doxorubicin, cyclophosphamide and prednisone (25). These regimens and rituximab combinations were chosen according to each institutional decision.

Statistical Analyses

Progression-free survival (PFS) was defined as the time between the date of initial chemotherapy until the date of PD or death from any cause. The absence of PD was treated as a censored observation. Overall survival (OS) was defined as the time from the date of initial chemotherapy to the date of death due to any cause. Patients without such events were treated as censored observations. PFS and OS were estimated using the Kaplan–Meier method, and survival curves were compared using the log-rank test. Univariate Cox regression analyses were used to estimate the hazard ratios and 95% confidence intervals. A two-sided $P < 0.05$ was considered to indicate statistical significance. Subgroups stratified by clinical variables were compared using the log-rank test. All statistical

analyses were performed using STATA 8.1 (STATACORP LP, College Station, TX, USA).

RESULTS

Patient Characteristics

Thirty-three patients with AIDS-BL were enrolled. The distributions of clinical characteristics of patients are listed in Table 1. Twenty cases underwent analysis of the *myc* gene, and *myc* rearrangements were detected in 14 patients. The median age was 41 (range 26–70) years, and 97% of the patients were male. Ten (30.3%) had a history of AIDS. The median CD4 + lymphocyte count and HIV viral load at diagnosis of AIDS-BL were 205/mm^3 (range 3–488/mm^3) and 13 700 copies/ml (range 0–12 000 000 copies/ml), respectively. Twenty-nine (87.9%) patients were diagnosed in the advanced stage (III/IV), with BM involvement in 17 (51.5%) patients and CNS infiltration in seven (21.2%) patients. Ten (30.3%) were treated with cART at diagnosis, and their median CD4 + lymphocyte count and HIV viral load at diagnosis of AIDS-BL were 316/mm^3 (range 3–488/mm^3) and 100 copies/ml (range 0–15,000 copies/ml), respectively. Viral load values were not available in two cases. Finally, cART was administered to 90.9% of the patients. For chemotherapy, six (18.2%) patients were treated with CODOX-M/IVAC, 23 (69.7%) patients were treated with hyper-CVAD/MA, two (6.1%) patients were treated with EPOCH and two (6.1%) patients were treated with CHOP.

Patient Outcomes

Response at the end of treatment among 32 assessable patients was as follows: ORR, 26 (78.8%) patients; CR, 24 (72.7%) patients; PR, two (6.1%) patients; SD, one (3.0%) patient and PD, five (15.2%) patients. The overall median follow-up period was 20.0 months (range 0.5–92.7 months). Figure 1 shows the Kaplan–Meier curves for PFS and OS in all patients. The estimated 2-year PFS and OS rates were 59.7 and 68.1%, respectively. At the time of analysis, 10 of 33 patients (30.3%) died, and nine (27.3%) experienced recurrence. There was one treatment-related death.

Impact of Rituximab and Chemotherapy Regimens

Twenty (60.6%) patients were treated with rituximab-containing regimens (rituximab group) and 13 (39.4%) patients were not (non-rituximab group). Of the 20 patients of the rituximab group, none were treated with CODOX-M/IVAC. Fifteen (75.0%) patients in the rituximab group and nine (69.2%) patients in the non-rituximab group achieved CR. The median follow-up in the rituximab group was 17.6 months (range 0.5–73.3 months) and that in the non-rituximab group was 34.1 months (range 1.3–92.7 months). The estimated 2-year PFS rates were 53.3% in the rituximab group and 69.2% in the non-rituximab group; the estimated

Table 1. Patient characteristics

	n	Percentage
Median age, years (range)	41 (26–70)	
≤30	3	9.1
31–40	12	36.4
41–50	10	30.3
51–60	6	18.2
≥61	2	6.1
Male sex	32	97.0
ECOG performance status		
0–1	11	33.3
≥2	22	66.7
Previous AIDS before AIDS-BL	10	30.3
Prior cART before AIDS-BL	10	30.3
Opportunistic disease at diagnosis of AIDS-BL	17	51.5
CD4 + lymphocyte count at diagnosis of AIDS-BL (/mm^3)		
<50	2	6.1
50–100	3	9.1
100–200	10	30.3
>200	18	54.5
Median HIV viral load, copies/ml (range)	13 700 (0–12 000 000)	
Ann Arbor stage		
I–II	4	12.1
III	2	6.1
IV	27	81.8
Bone marrow involvement	17	51.5
CNS infiltration	7	21.2
Extranodal disease	30	90.9
Serum LDH > upper limit of normal	27	81.8
Chemotherapy regimen		
CODOX-M/IVAC	6	18.2
Hyper-CVAD/MA	23	69.7
EPOCH	2	6.1
CHOP	2	6.1
Rituximab		
Yes	20	60.6
No	13	39.4

n, number of patients; ECOG, Eastern Cooperative Oncology Group; AIDS, acquired immunodeficiency syndrome; AIDS-BL, AIDS-related Burkitt lymphoma; cART, combination antiretroviral therapy; HIV, human immunodeficiency virus; CNS, central nervous system; LDH, lactate dehydrogenase; CODOX-M/IVAC, cyclophosphamide, vincristine, doxorubicin, dexamethasone, etoposide, ifosfamide and cytarabine; hyper-CVAD/MA, cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine; EPOCH, etoposide, vincristine, doxorubicin, cyclophosphamide and prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone

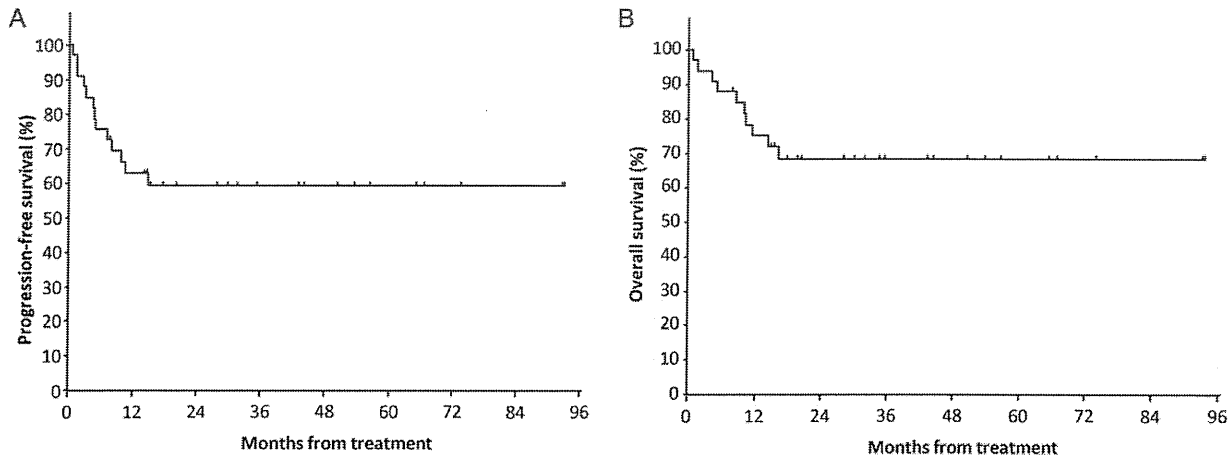

Figure 1. Kaplan–Meier curve of progression-free survival (PFS) (A) and overall survival (OS) (B) in patients with acquired immunodeficiency syndrome-Burkitt lymphoma.

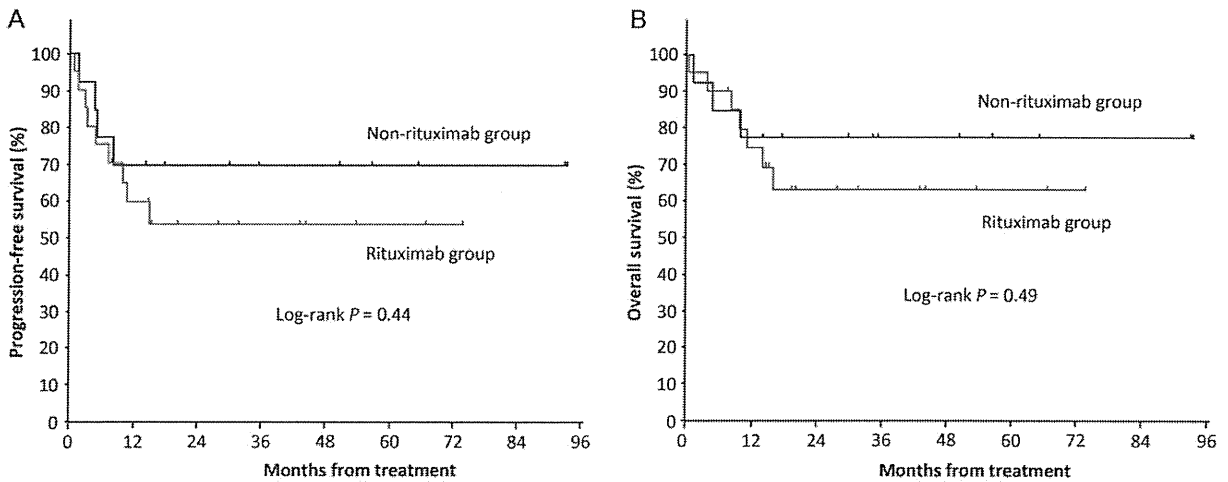

Figure 2. Kaplan–Meier curve of PFS (A) and OS (B) in patients with acquired immunodeficiency syndrome-Burkitt lymphoma treated by rituximab -containing chemotherapy (rituximab group: gray line) and by chemotherapy alone (non-rituximab group: black line).

2-year OS rates were 62.6 and 76.9%, respectively. There was no significant difference in PFS and OS between chemotherapy regimens with rituximab and without rituximab ($P = 0.44$ and $P = 0.49$, respectively) (Fig. 2).

Six (18.2%) patients received CODOX-M/IVAC and 22 (66.7%) patients received hyper-CVAD/MA. The patients receiving rituximab was none in the CODOX-M/IVAC group and 18 (81.8%) in the hyper-CVAD/MA group. Age, PS, clinical stage and LDH were similar among the CODOX-M/IVAC and hyper-CVAD/MA groups. We also compared the PFS and the OS between patients receiving CODOX-M/IVAC and patients receiving hyper-CVAD/MA. The median follow-up in patients receiving CODOX-M/IVAC was 21.9 months (range 1.3–92.7 months) and that in patients receiving hyper-CVAD/MA was 27.7 months (range 0.5–73.3 months). The 2-year PFS rate was 66.7% for patients receiving CODOX-M/IVAC, compared with 64.2% for patients receiving hyper-CVAD/MA ($P = 0.35$) (Fig. 3). The 2-year OS rate was 66.7% for patients receiving CODOX-M/IVAC, compared with 72.6% for patients receiving hyper-CVAD/MA ($P = 0.72$).

Prognostic Factors for Survival in Patients with AIDS-BL

Significant clinical variables that affected PFS or OS were identified using univariate Cox regression analyses. Factors predicting poor PFS in univariate analyses were CNS infiltration, extranodal disease (≥ 2), IPI score (3–4) and CR to chemotherapy ($P = 0.030$, $P = 0.039$, $P = 0.013$ and $P = 0.023$, respectively). There was no significant prognostic factor for OS in univariate analyses.

The clinical outcomes were compared between MYC positive cases ($n = 14$) and negative cases ($n = 6$). There was no significant difference in OS and PFS between the abnormalities and non-abnormalities of MYC ($P = 0.16$ and $P = 0.19$, respectively).

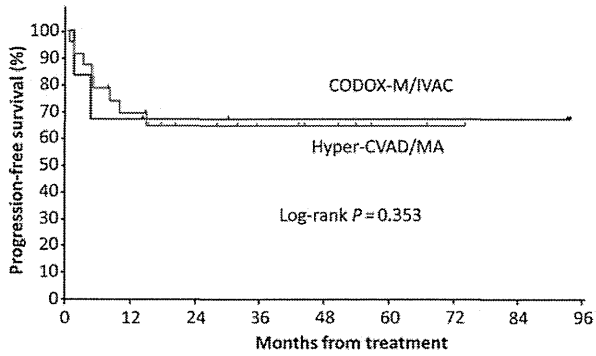

Figure 3. Kaplan–Meier curve of PFS in patients with acquired immunodeficiency syndrome-Burkitt lymphoma treated by hyper-CVAD/MA (gray line) and by CODOX-M/IVAC (black line).

DISCUSSION

We retrospectively evaluated the clinical outcomes of patients with AIDS-BL who received chemotherapy. We identified 33 patients who were treated at regional hospitals for HIV/AIDS in Japan. The ORR and CR rates for all patients were 79 and 73%, respectively. The estimated 2-year PFS and OS rates of all patients were 59.7 and 68.1%, respectively. Mead et al. (23) conducted a clinical trial of highly intensive regimens for non-HIV BL and AIDS-BL, and reported that the estimated 2-year PFS and OS rates of all patients were 60 and 63%, respectively. Thus, favorable overall outcomes were shown for patients with AIDS-BL in this study. There was no significant difference in the OS and the PFS between chemotherapy regimens with/without rituximab ($P = 0.49$ and $P = 0.44$, respectively). In addition, there were no differences in the OS and the PFS when comparing CODOX-M/IVAC and hyper-CVAD/MA groups ($P = 0.72$ and $P = 0.35$, respectively).

The institution of cART has transformed HIV infection into a chronic disease in developed countries and has decreased the incidence of ADCs (26–28). cART consists of two or three antiretroviral agents including reverse transcriptase inhibitors (nucleoside/nucleotide, non-nucleoside), protease inhibitors and integrase strand transfer inhibitor. The application of cART and the cautious management of infections (including prevention) have significantly improved the prognosis of the HIV-infected population, mostly by reducing opportunistic infections. However, the incidence of BL and cervical cancer has not declined in this population (9, 26). AIDS-BL tended to occur in patients with relatively higher CD4 + lymphocyte counts when compared with other lymphomas (3, 5–10). Chronic antigenic stimulation of B-cell by chronic HIV viremia may be the pathogenetic mechanism of AIDS-BL, and patients with chronic HIV viremia have a higher incidence of ARL than those with undetectable viral loads (28, 29). In the present study, 10 (30.3%) patients had been treated with cART at the time of AIDS-BL diagnosis, and the viral loads of these patients tended to be low. Two patients had undetectable viral loads. Therefore, even with the widespread use of cART, AIDS-BL remains an important disease among ADCs.

BL is a rapidly proliferating tumor that develops over a matter of days or weeks and that has a propensity for CNS involvement. The prognosis of patients with AIDS-BL is very poor. Lim et al. (11) reported that the addition of cART to CHOP-based chemotherapy resulted in a significant improvement in the outcome of AIDS-related DLBCL, whereas the survival of patients with AIDS-BL remained poor. Similarly, in a Phase II study, the survival of patients with AIDS-BL was significantly worse when compared with patients of AIDS non-BL who were treated with rituximab plus CDE (cyclophosphamide, doxorubicin and etoposide) (30). The reason for these poor outcomes was likely related to the fact that patients received only reduced-dose chemotherapy regimens (13, 14). Wang et al. (31) compared HIV-infected BL patients and non-HIV-infected BL patients treated with CODOX-M/IVAC or less intensive regimens, and reported that the 2-year event-free survival was significantly better in patients treated with CODOX-M/IVAC than in those receiving less intensive chemotherapy. In two retrospective studies, toxicity and outcomes experienced by patients with AIDS-BL were similar to those of patients with non-HIV BL in response to CODOX-M/IVAC or hyper-CVAD/MA (24, 31). Another prospective study clarified that patients with AIDS-BL treated with intensive immunochemotherapy had a higher incidence of severe mucositis and infections than patients with non-HIV-infected BL; however, the survival was similar when comparing those two groups (32). Therefore, in the cART era, more intensive regimens should be considered for patients with AIDS-BL in a manner similar to treatment offered to patients with non-HIV BL. EPOCH would be another promising regimen for ARLs including BL and be less intensive than CODOX-M/IVAC and hyper-CVAD/MA (25, 33). It might be considered a good alternative for frail patients.

Standard chemotherapy regimen for AIDS-BL patients remains controversial. Our study showed that the type of chemotherapy regimen had no significantly different impact on outcomes when comparing CODOX-M/IVAC vs. hyper-CVAD/MA (2-year PFS, 66.7 and 64.2%, respectively; $P = 0.35$, and 2-year OS, 66.7 and 72.6%, respectively; $P = 0.72$). This result suggests that intensive chemotherapy regimens might provide to favorable outcomes to patients with AIDS-BL.

One important question is whether patients with AIDS-BL should be treated with rituximab. The B-cell lineage restricted marker, CD20 is strongly expressed in BL. *In vitro*, rituximab has a proven anti-BL effect (34). One randomized clinical trial of ARL treated with rituximab reported that the addition of rituximab to CHOP was associated with a significantly higher risk of treatment-related death when compared with the use of CHOP alone. This was due to an increase in infection-related deaths in patients receiving rituximab plus chemotherapy, especially in patients with CD4 + lymphocyte counts $<50/mm^3$ (35). Another study showed that the addition of rituximab increased the risk of infections (36). No randomized trial examining the role of rituximab has been performed in patients with

AIDS-BL. In the present study, rituximab-containing chemotherapy was not superior to chemotherapy without rituximab. The reasons for these results were not apparent, but the rituximab-containing highly intensive chemotherapy might contain some defects in treating AIDS-BL patients.

In conclusion, this study demonstrated favorable overall outcomes for patients with AIDS-BL in the cART era. Highly intensive chemotherapy regimens would bring high remission rates and prolonged OS. The addition of rituximab to highly intensive chemotherapy has not shown to be beneficial for patients with AIDS-BL. These data warrant the design of a prospective trial to optimize the treatment for patients with AIDS-BL.

Funding

This study was supported by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour and Welfare of Japan (grant number: H22-AIDS-002).

Conflict of interest statement

None declared.

References

1. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. *Blood* 2004;104:3009–20.
2. Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. *Lancet* 1991;337:805–9.
3. Straus DJ. Treatment of Burkitt's lymphoma in HIV-positive patients. *Biomed Pharmacother* 1996;50:447–50.
4. Newcom SR. Acquired immunodeficiency syndrome-related lymphoma. *Blood* 1992;80:2944–6.
5. Molyneux EM, Rochford R, Griffin B, et al. Burkitt's lymphoma. *Lancet* 2012;379:1234–44.
6. Besson C, Goubar A, Gabarre J, et al. Changes in AIDS-related lymphoma since the era of highly active antiretroviral therapy. *Blood* 2001;98:2339–44.
7. Pluda JM, Venzon DJ, Tosato G, et al. Parameters affecting the development of non-Hodgkin's lymphoma in patients with severe human immunodeficiency virus infection receiving antiretroviral therapy. *J Clin Oncol* 1993;11:1099–107.
8. Carbone A, Gloghini A, Gaidano G, et al. AIDS-related Burkitt's lymphoma. Morphologic and immunophenotypic study of biopsy specimens. *Am J Clin Pathol* 1995;103:561–7.
9. Mounier N, Spina M, Gabarre J, et al. AIDS-related non-Hodgkin lymphoma: final analysis of 485 patients treated with risk-adapted intensive chemotherapy. *Blood* 2006;107:3832–40.
10. Guech-Ongey M, Simard EP, Anderson WF, et al. AIDS-related Burkitt lymphoma in the United States: what do age and CD4 lymphocyte patterns tell us about etiology and/or biology? *Blood* 2010;116:5600–4.
11. Lim ST, Karim R, Nathwani BN, Tulpule A, Espina B, Levine AM. AIDS-related Burkitt's lymphoma versus diffuse large-cell lymphoma in the pre-highly active antiretroviral therapy (HAART) and HAART eras: significant differences in survival with standard chemotherapy. *J Clin Oncol* 2005;23:4430–8.
12. Spina M, Simonelli C, Talamini R, Tirelli U. Patients with HIV with Burkitt's lymphoma have a worse outcome than those with diffuse large-cell lymphoma also in the highly active antiretroviral therapy era. *J Clin Oncol* 2005;23:8132–3; author reply 3–4.
13. Dave SS, Fu K, Wright GW, et al. Molecular diagnosis of Burkitt's lymphoma. *N Engl J Med* 2006;354:2431–42.
14. Bishop PC, Rao VK, Wilson WH. Burkitt's lymphoma: molecular pathogenesis and treatment. *Cancer Invest* 2000;18:574–83.
15. Xicoy B, Ribera JM, Miralles P, et al. Comparison of CHOP treatment with specific short-intensive chemotherapy in AIDS-related Burkitt's lymphoma or leukemia. *Med Clin (Barc)* 2011;136:323–8.
16. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood* 2005;105:1417–23.
17. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346:235–42.
18. Habermann TM, Weller EA, Morrison VA, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol* 2006;24:3121–7.
19. Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981;47:207–14.
20. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res* 1971;31:1860–1.
21. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med* 1993;329:987–94.
22. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol* 1999;17:1244.
23. Mead GM, Barrans SL, Qian W, et al. A prospective clinicopathologic study of dose-modified CODOX-M/IVAC in patients with sporadic Burkitt lymphoma defined using cytogenetic and immunophenotypic criteria (MRC/NCRI LY10 trial). *Blood* 2008;112:2248–60.
24. Cortes J, Thomas D, Rios A, et al. Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone and highly active antiretroviral therapy for patients with acquired immunodeficiency syndrome-related Burkitt lymphoma/leukemia. *Cancer* 2002;94:1492–9.
25. Sparano JA, Lee JY, Kaplan LD, et al. Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated B-cell non-Hodgkin lymphoma. *Blood* 2010;115:3008–16.
26. Shiels MS, Pfeiffer RM, Hall HI, et al. Proportions of Kaposi sarcoma, selected non-Hodgkin lymphomas, and cervical cancer in the United States occurring in persons with AIDS, 1980–2007. *JAMA* 2011;305:1450–9.
27. Bedimo R, Chen RY, Accortt NA, et al. Trends in AIDS-defining and non-AIDS-defining malignancies among HIV-infected patients: 1989–2002. *Clin Infect Dis* 2004;39:1380–4.
28. Shiels MS, Cole SR, Wegner S, et al. Effect of HAART on incident cancer and noncancer AIDS events among male HIV seroconverters. *J Acquir Immune Defic Syndr* 2008;48:485–90.
29. Zoufaly A, Stellbrink HJ, Heiden MA, et al. Cumulative HIV viremia during highly active antiretroviral therapy is a strong predictor of AIDS-related lymphoma. *J Infect Dis* 2009;200:79–87.
30. Spina M, Jaeger U, Sparano JA, et al. Rituximab plus infusional cyclophosphamide, doxorubicin, and etoposide in HIV-associated non-Hodgkin lymphoma: pooled results from 3 phase 2 trials. *Blood* 2005;105:1891–7.
31. Wang ES, Straus DJ, Teruya-Feldstein J, et al. Intensive chemotherapy with cyclophosphamide, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, and high-dose cytarabine (CODOX-M/IVAC) for human immunodeficiency virus-associated Burkitt lymphoma. *Cancer* 2003;98:1196–205.
32. Oriol A, Ribera JM, Bergua J, et al. High-dose chemotherapy and immunotherapy in adult Burkitt lymphoma: comparison of results in human immunodeficiency virus-infected and noninfected patients. *Cancer* 2008;113:117–25.
33. Little RF, Pittaluga S, Grant N, et al. Highly effective treatment of acquired immunodeficiency syndrome-related lymphoma with dose-adjusted EPOCH: impact of antiretroviral therapy suspension and tumor biology. *Blood* 2003;101:4653–9.
34. Daniels I, Abulayha AM, Thomson BJ, Haynes AP. Caspase-independent killing of Burkitt lymphoma cell lines by rituximab. *Apoptosis* 2006; 11:1013–23.
35. Kaplan LD, Lee JY, Ambinder RF, et al. Rituximab does not improve clinical outcome in a randomized phase 3 trial of CHOP with or without rituximab in patients with HIV-associated non-Hodgkin lymphoma: AIDS-Malignancies Consortium Trial 010. *Blood* 2005;106:1538–43.
36. Ribera JM, Oriol A, Morgades M, et al. Safety and efficacy of cyclophosphamide, adriamycin, vincristine, prednisone and rituximab in patients with human immunodeficiency virus-associated diffuse large B-cell lymphoma: results of a phase II trial. *Br J Haematol* 2008; 140:411–9.

EXPERT OPINION

New approaches to treating primary effusion lymphoma

Hiroki Goto & Seiji Okada†
†*Kumamoto University, Center for AIDS Research, Division of Hematopoiesis, Chuo-ku, Kumamoto, Japan*

Introduction: Primary effusion lymphoma (PEL) is rare and aggressive B-cell non-Hodgkin's lymphoma that presents with malignant effusions without tumor masses. Kaposi's sarcoma-associated herpes virus/human herpes virus-8 is universally associated with the pathogenesis of PEL. It is generally resistant to chemotherapy with a short median survival of < 6 months. The optimal treatment for PEL is not well defined and there is a need to establish a standard therapy.

Areas covered: This review summarizes the disease features, pathogenesis, diagnosis and current and new targeted treatments for PEL. A literature search on PubMed has been undertaken and the most relevant references have been considered.

Expert opinion: Initiating or continuing combination antiretroviral therapy is recommended for HIV-infected PEL patients; however, there is no standard chemotherapy for PEL due to its low incidence and the lack of a large clinical study. PEL is resistant to conventional cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP)-like chemotherapy. Thus, therapeutic approaches that target aggressive and chemotherapy-resistant nature in PEL are considered for treatment. In the use of novel agents, further clinical studies on resistant hematological malignancy, such as multiple myeloma, would provide evidence for their clinical use.

Keywords: combination antiretroviral therapy, human immunodeficiency virus, Kaposi's sarcoma-associated herpes virus, molecular targeted therapy, mouse model, primary effusion lymphoma

Expert Opinion on Orphan Drugs [Early Online]

1. Introduction

Primary effusion lymphoma (PEL) is a rare aggressive subtype of non-Hodgkin's lymphoma (NHL) that typically shows malignant effusions without tumor masses. Human immunodeficiency virus (HIV)-infected individuals have a 60 – 200 times higher relative risk of developing NHL than the HIV-negative population [1]. Among HIV-associated lymphoma, PEL arises more frequently in the HIV-infected population. PEL accounts for approximately 4% of all HIV-associated NHL cases [2]. PEL was first described in 1989 as an AIDS-related lymphoma of uncertain lineage that demonstrated B-cell derivation and included Epstein–Barr virus (EBV) [3]. In 1995, Cesarman *et al.* identified Kaposi's sarcoma-associated herpes virus (KSHV) DNA sequences within a distinct subtype of AIDS-related lymphoma presenting with lymphomatous effusions [4]. In 1996, Nador *et al.* designated this lymphoma as 'primary effusion lymphoma', which is a distinct entity associated with KSHV [5]. KSHV, also called human herpes virus-8 (HHV-8), is a member of the γ-herpes virus family with a peculiar tropism for lymphocytes, endothelial cells, keratinocytes and marrow stromal cells. KSHV was first characterized in HIV-infected patients with Kaposi's sarcoma (KS) [6]. KSHV-infected endothelial cells constitute KS. KSHV also causes a lymphoproliferative disorder, multicentric

10.1517/21678707.2013.852467 © 2013 Informa UK, Ltd. e-ISSN 2167-8707

Article highlights.

- Primary effusion lymphoma (PEL) is universally associated with KSHV/HHV-8 infection and demonstrates unique clinical features.
- Detection of KSHV LANA-1 and phenotypic analysis lead to a diagnosis of PEL.
- Standard treatment of PEL is not well defined due to its low incidence and lack of clinical trials.
- Initiation or continuation of cART and supportive therapies are recommended at the onset of diagnosis.
- Molecular-targeted therapies, including targeting proteasome, cytokines and surface antigens, are being considered for PEL treatment.

This box summarizes key points contained in the article.

Castleman's disease (MCD) by infecting plasmablasts expressing IgM lambda [7]. In the majority of PEL cases, co-infection with EBV has been detected. EBV plays an unclear role in PEL pathogenesis. In the World Health Organization classification of neoplastic diseases of hematopoietic and lymphoid tissues, PEL is described as a distinct entity and is also included in 'lymphomas occurring more specifically in HIV-positive patients' among HIV-associated lymphoma [8].

Given its pathogenesis and unique clinical manifestation, the clinical evidence observed in HIV-associated lymphoma, including diffuse large B-cell lymphoma (DLBCL) and Burkitt's lymphoma (BL), could not be applied to PEL. Conventional cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) regimens did not improve survival compared with other HIV-associated NHL. Survival on conventional chemotherapy is very poor with PEL. The effusions can be managed by repeated drainage but eventually solid nests of PEL abolish vital organ functions. A large clinical trial has not been possible for PEL until now due to the low incidence of this lymphoma. To assess the *in vivo* efficacy of novel agents, a PEL xenograft mouse model could be a useful tool because the mouse model showed ascites formation, reflecting the clinical manifestation of human PEL [9,10].

In this review, the therapeutic evidence from case series and the potential use of drugs and novel therapeutic approaches from preclinical evaluation using a mouse model of this lymphoma are discussed.

2. Primary effusion lymphoma

2.1 Clinical features

PEL is clinically characterized by lymphomatous effusions in body cavities, including the pleural, pericardial and peritoneal cavities, usually without extracavitary tumor masses. Patients present with dyspnea from pleural or pericardial effusion or abdominal distension from ascites, which are the results of mass effects of malignant effusions. PEL usually occurs in advanced AIDS patients with a decreased CD4 T-cell count at diagnosis. About 50% of the PEL patients have preexisting KS or develop KS [11]. HIV-negative PEL patients have been described in elderly men from the Mediterranean region and in immunocompromised patients after solid organ transplantation [12,13].

The incidence of PEL depends on the seroprevalance of KSHV. There are endemic areas of KSHV infection, including sub-Saharan Africa (infection rate of 50 – 70%) and the Mediterranean region (infection rate of 20 – 30%), while North America exhibits only a 1 – 3% infection rate among healthy donors [14]. However, KSHV infection is not likely sufficient. The presence of cofactors such as an immunodeficient status may hasten the progression to disease.

Recently, PEL has been categorized into classic PEL and an extracavitary variant of PEL. Classic PEL is characterized by lymphomatous effusions in body cavities, while an extracavitary variant of PEL present as a solid tumor and have no malignant effusions. Their morphology, immunophenotypes and molecular characteristic are not significantly different [15].

Classification of KSHV-associated diseases, including PEL, KSHV-associated MCD and KS, is shown in Table 1.

2.2 Laboratory features

Morphologically, PEL cells show features of immunoblastic lymphoma and anaplastic large-cell lymphoma (ALCL). They also show plasma cell differentiation (Figure 1).

PEL cells express plasma-cell markers (CD38, CD138 and IRF4/MUM1), CD30, CD71 and epithelial membrane antigen, human leukocyte antigen-DR and CD45 [5]. B-cell markers (CD19, CD20, CD79a, surface and cytoplasmic immunoglobulin) and T-cell markers (CD2, CD3, CD4 CD5, CD7 and CD8) are not typically detected. Despite its null phenotype, PEL is represented by a monoclonal B-cell population.

Immunohistochemistry for latent nuclear antigen-1 (LANA-1) is recommended to detect the presence of KSHV in lymphoma cells. Polymerase chain reaction amplification using a DNA extract from lymphoma cells is also useful in detecting KSHV and in measuring peripheral blood KSHV viral load [16]. Evidence of EBV infection is most reliably detected by *in situ* hybridization for EBV-encoded small RNA (EBER), while immunohistochemical staining for EBV latent membrane protein-1 is negative.

High levels of interleukins (IL-6 and IL-10) and soluble forms of antigens, such as soluble CD30, might also help in diagnosis [17,18].

Cytogenetic analysis has demonstrated complex karyotypes but no common chromosomal abnormality. Analysis of immunoglobulin gene rearrangement shows monoclonality of B-cell origin.

2.3 Molecular genetics

PEL cells contain 40 – 80 copies of KSHV episomes per cell and express KSHV latent genes. Four latent gene products, which are thought to play significant roles in PEL, are LANA-1, viral cyclin (v-cyclin), viral FADD-like

Table 1. Classification of KSHV-associated diseases.

	PEL	KSHV-associated MCD	KS
Disease	Lymphoma	Lymphoproliferative disease	Sarcoma
Cell origin	Post-germinal center B cell	Naïve B cell	Blood vascular endothelial cell
Organ	Body cavity (classic PEL)	Lymph node	Skin, internal organ
EBV	+ (50 – 80%)	-	-

KS: Kaposi's sarcoma; MCD: Multicentric Castleman's disease; PEL: Primary effusion lymphoma.

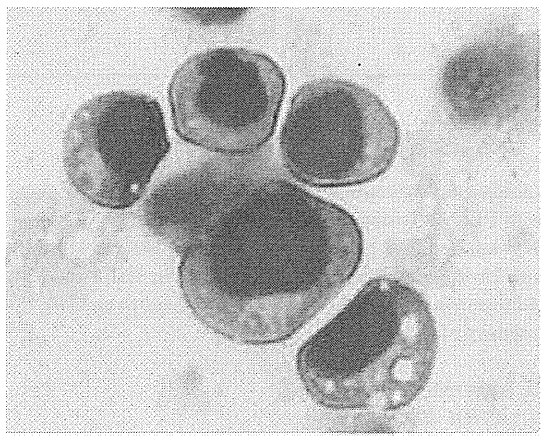

Figure 1. Wright–Giemsa staining of PEL cells from a patient with pericardial effusion are shown. PEL cells show features of immunoblastic lymphoma and ALCL characterized by large size, large nucleus with prominent nucleoli and abundant basophilic cytoplasm.

interleukin-1 β-converting enzyme (FLICE-inhibitory protein) (vFLIP) and Kaposin. Additional latency-associated proteins assigned to PEL include vIL-6 and LANA-2/vIRF-3. LANA-1 binds to TP53 and RB1, impairing apoptosis of KSHV-infected cells [19,20]. The v-cyclin, which is a viral homolog of cyclin D, binds to cyclin-dependent kinase 6, and induces progression through the cell cycle [21]. The vFLIP inhibits apoptosis by blocking Fas-mediated caspase activation and activates nuclear factor-kappa B (NF-κB) [22,23]. Kaposin A has oncogenic potential through cytokesin-1 [24]. Kaposin B stabilizes cytokine expressions, such as IL-6 and granulocyte-macrophage colony-stimulating factor, by inhibiting the degradation of mRNA containing AU-rich elements [25]. The vIL-6 is a homolog of cellular IL-6 (24.6% amino acid sequence identity) and its signaling triggers the Janus tyrosine kinases/signal transducers and activators of transcription (JAK/STAT) pathway, thus inducing autocrine/paracrine signaling [26]. LANA-2/vIRF-3 has a potential role in developing drug resistance by binding to polymerized microtubules, reducing their stability [27]. Further, KSHV encodes homologous human interferon response factors (IRF), which inhibit interferon (IFN)-mediated effects [28]. The major latency-associated region of KSHV genome encodes 12 microRNA (miRNA) genes. Of note, miR-K12-11 is a KSHV miRNA sharing full seed sequence homology with human miRNA, miR-155. Given that miR-155 promotes plasma cell differentiation, miR-K12-11 might contribute to KSHV lymphomagenesis [29,30].

Approximately 50 – 80% of PEL are co-infected with EBV [5]. EBV gene expression in dually infected PEL cells is restricted to Epstein–Barr nuclear antigen 1 and EBER. Although EBV-positive PEL exhibits a different pattern of gene expression compared to EBV-negative PEL, there is no evidence that EBV-positive PEL presents with the characteristic clinical manifestation.

Immunoglobulin gene rearrangements and somatic hypermutation occur in PEL cells [31]. Gene expression analysis has shown a phenotype intermediate between immunoblasts and plasma cells [32]. These results suggest that the cell of origin is a post-germinal center B cell.

Most of the cases lack the characteristic chromosomal translocation, such as BCL-2, BCL-6 and c-MYC, and show complex karyotypes without recurrent abnormalities [4,5]. In PEL cell lines, p53 and PTEN genes are rarely mutated. The fragile histidine triad and WW domain-containing oxidoreductase, two fragile site tumor suppressor genes, are deleted in many PEL cell lines (85%) [33]; however, genome-wide association analysis is limited by the scarcity of cases.

2.4 Differential diagnosis

The most common differential diagnosis in cases of PEL is another type of NHL with lymphomatous effusion, such as DLBCL and BL with secondary effusion. Confirmation of the typical morphology and immunophenotype described previously and evidence of KSHV infection are required for the diagnosis of PEL.

Pyothorax-associated lymphoma (PAL), which arises in the setting of long-standing pyothorax related to the treatment of tuberculosis, may be included in the differential diagnosis. PAL is more common in Japan and usually occurs in elderly men with a history of pulmonary tuberculosis or tuberculous pleuritis treated with pneumothorax, resulting in a pyothorax. This lymphoma is usually a large cell lymphoma of B-cell lineage, expressing B-cell markers and staining EBV-positive and HHV-8 negative [34].

To date, 52 cases of KSHV/HHV-8-unrelated PEL-like lymphoma have been reported in the literature [35]. Recently,

Table 2. Laboratory features of classic PEL and other lymphomas with effusion.

	PEL	DLBCL	BL	KSHV-unrelated PEL-like lymphoma
Effusion	Primary	Secondary	Secondary	Primary
KSHV	++	-	-	-
		-/+	+	
EBV	+ (50 – 80%)	(HIV-associated: 30 – 90%)	(HIV-associated: 30 – 70%)	+ (30%)
CD19, CD20	-	+	+	+ (70 – 80%)
CD30	+	-	-	-
CD138	+	-	-	-
c-*myc* rearrangement	-	-	+	-

++: 100%; +: 20%-99%; -: < 20%.
BL: Burkitt's lymphoma; DLBCL: Diffuse large B-cell lymphoma; PEL: Primary effusion lymphoma.

the term 'KSHV/HHV8-negative effusion-based lymphoma' was proposed by Alexanian *et al.* [36]. Similar to PEL, this lymphoma presents with lymphomatous effusion without detectable masses. KSHV is negative in all cases. Hepatitis C virus and EBV are positive in nearly 30% of cases, respectively. Patients are generally elderly and have underlying medical conditions, such as cirrhosis or cardiovascular dysfunction. It is considered to be associated with fluid overload states. Laboratory features of classic PEL and other lymphomas with effusion are shown in Table 2.

Plasmablastic lymphoma (PBL) is an infrequent B-cell NHL with a common extranodal presentation in HIV-infected patients. The involvement of the oral cavity is most frequently associated with PBL but has also been reported in other organs such as the gastrointestinal tract. The prognosis of PBL is as poor as that of PEL. The morphology shows plasmablastic differentiation. The immunophenotype of PBL is similar to that of PEL ($CD20^-$, $CD38^+$ and $CD138^+$). KSHV-positive solid lymphomas with plasmablastic features should be considered an extracavitary variant of PEL.

Because of their similar morphology and lack of a B-cell marker, T-cell ALCL is sometimes confused with PEL [37]. Immunohistochemistry for anaplastic lymphoma kinase and the T-cell receptor gene rearrangement would be helpful in these cases.

The differential diagnosis of PEL requires morphological studies by an expert pathologist and analysis of virology and phenotypes. Among these examinations, the presence of KSHV infection is necessary in the diagnosis of PEL. Suspecting PEL and performing LANA-1 immunohistochemistry play the most important roles in diagnosis.

2.5 Prognostic factors and outcome

All cases of PEL are stage IV in standard Ann Arbor staging due to the presence of malignant effusions in body cavities. The International Prognostic Index, which predicts prognosis in NHL [38], has never been assessed in a large cohort of PEL patients. Poor performance status and the absence of combination antiretroviral therapy (cART) before the diagnosis of PEL are strong predictors of a poor clinical outcome in HIV-infected PEL patients [2,39]. The number and location of body cavities involved are also prognostic in PEL patients [40]. Multicentric cooperative studies are required to confirm these prognostic factors.

2.6 Treatment

2.6.1 *CHOP-like regimen*

CHOP consists of cyclophosphamide, doxorubicin, vincristine and prednisone. CHOP chemotherapy is one of the most common chemotherapy regimens for treating NHL. This regimen can also be combined with a chimeric anti-CD20 monoclonal antibody rituximab if the lymphoma is of B-cell origin expressing CD20 (R-CHOP); however, rituximab is not incorporated into the treatment regimen in PEL because this lymphoma generally lacks CD20. A CHOP-like regimen has been reported to achieve a 43 – 57% complete remission (CR) rate in PEL patients; however, overall survival is still < 6 months [2,39,41]; therefore, there is a need to develop new regimens to improve the clinical outcome of PEL instead of CHOP.

2.6.2 *Combination antiretroviral therapy*

Prior to the administration of cART, the therapeutic results with chemotherapy containing anthracycline were unsatisfactory in HIV-positive patients. The prognostic impact of cART in combination with chemotherapy has been reported in PEL [39]. In addition, CR of PEL patients with sole cART has been described [42,43]. Thus, implementation of cART is recommended when treating HIV-infected patients with PEL. Among antiretroviral agents, protease inhibitors may modify the metabolism of cytotoxic drugs and potentiate myelotoxicity by inhibiting the CYP3A4 enzyme [44]. Thus, anticancer drugs, which rely on CYP, should be used carefully with protease inhibitor-based regimens to avoid inadvertent toxicity. Raltegravir-based regimens could be the first choice during chemotherapy because of less frequent drug interactions.

2.6.3 Proteasome inhibitor

Bortezomib is a 26S proteasome inhibitor that is effective for several malignancies, such as multiple myeloma [45] and mantle cell lymphoma [46]. Bortezomib can induce apoptosis via several mechanisms, including NF-κB inhibition [47], activation of a terminal unfolded protein response [48], and prevention of degradation of proapoptotic proteins and tumor-suppressor genes targeted for ubiquitination by KSHV during viral oncogenesis [49]. In clinical salvage therapy in three HIV-infected PEL patients, bortezomib failed to control the progression of PEL [50]. Bortezomib has been shown to enhance the *in vitro* antitumor effect of anticancer agents such as doxorubicin [51]; therefore, combination chemotherapy with bortezomib and anthracycline-based regimens is promising.

2.6.4 Inhibition of NF-κB

The vFLIP has the ability to activate the NF-κB pathway by binding to the IκB kinase (IKK) complex [22,23]. Activation of NF-κB is involved in various kinds of cancer development and progression [52]. NF-κB activation is also critical for KSHV viral latency and oncogenesis. Although recent reports showed that bortezomib did not inhibit NF-κB activity [53,54], NF-κB could be an effective molecular target of PEL [55-58]. The development of NF-κB inhibitors that have low toxicity is expected. Recently, the effects of heat shock protein 90 (HSP90) inhibitors on the proliferation of PEL cells via the inhibition of the NF-κB pathway have been reported [59,60]. The molecular chaperone HSP90 could be a molecular target because it is a component of the IKK complex and is required for its activity.

2.6.5 JAK inhibitor

Constitutive phosphorylation of JAK2/STAT3 is constitutively phosphorylated in PEL cell lines [61]. The JAK2 inhibitor AG490 suppressed STAT3 phosphorylation, resulting in efficient apoptosis and the reduction of survivin expression. Survivin has been implicated in both cell cycle control and apoptosis resistance [62]. Targeting survivin by STAT3 inhibition may provide an effective therapeutic approach for PEL. Curcumin (diferuloylmethane), a natural compound isolated from the plant *Curcuma longa*, also suppresses STAT3 activation, inducing the apoptosis of PEL cells [63]. Several JAK2 inhibitors, which are currently undergoing clinical trials in patients with myeloproliferative neoplasms, are also promising in the treatment of PEL.

2.6.6 Inhibition of phosphoinositol-3-kinase/AKT

The phosphoinositol-3-kinase (PI3K)/AKT pathway is constitutively activated in PEL cell lines [64]. KSHV K1 activates the PI3K/AKT pathway in B cells [65]. Another viral protein, viral G-protein-coupled receptor, can also activate this pathway in endothelial and epithelial cells [66]. No activating PI3K mutations have been detected in PEL cell lines, and only two PEL cell lines display PTEN mutations associated with a loss of PTEN protein expression [67]. Inhibition of PI3K activity by LY294002 causes dephosphorylation of AKT, GSK3 and FKHR, inducing apoptosis in PEL cell lines [64]. Suppression of p-AKT in PEL cells via generation of reactive oxygen species is also effective for PEL treatment [68]. Moreover, there is a strong biological link between NF-κB and PI3K/AKT in the modulation of anti-apoptotic effects in PEL cells [69]. Synergistic targeting of NF-κB and PI3K/AKT pathways may have a therapeutic potential for the treatment of PEL. PI3K pathway inhibitors are already undergoing early clinical trials for several malignancies, providing important implications for the treatment of PEL.

2.6.7 Inhibition of mammalian target of rapamycin

Mammalian target of rapamycin (mTOR), its activator AKT and its target p70S6 kinase are phosphorylated in PEL cell lines [70]. The allosteric mTORC1 inhibitor rapamycin binds to FK506-binding protein 12, inhibiting mTOR kinase activity. Rapamycin has been effective at inducing apoptosis of PEL cell lines and in an *in vivo* xenograft mouse model [70]; however, two renal transplant patients who received rapamycin have been reported to develop PEL. Rapamycin may not protect HHV-8-infected renal transplant recipients from the occurrence of PEL or progression of preexisting PEL [71]. The dual PI3K/mTOR inhibitor NVP-BEZ235 has been significantly more efficacious *in vitro* and in a PEL xenograft tumor model. AKT at Ser473 is phosphorylated by the rapamycin-insensitive mTORC2, which is responsible for activating AKT in a feedback loop and has been implicated in rapamycin failure after prolonged treatment [72,73]. NVP-BEZ235 can prevent this feedback loop.

2.6.8 Antiviral therapy

Antiviral treatment can be induced to effect the lytic phase of KSHV viral replication. CR has been reported after the administration of antiviral nucleotide analog, cidofovir [43,74,75], an antiviral agent with broad activity against multiple DNA viruses, inducing lytic replication of KSHV. *In vitro* studies have also shown the efficacy of antiviral therapy against PEL cell lines [76]. Induction of lytic replication of KSHV while blocking virus production has been considered for anti-PEL therapy [77].

2.6.9 Combination therapy with IFN-α and azidothymidine

IFNs were found to induce the expression of the proapoptotic protein tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in human dendritic cells and T lymphocytes, which caused apoptosis in TRAIL receptor-expressing targets [78,79]. Azidothymidine (AZT), which is also known as the first HIV nucleoside reverse-transcriptase inhibitor, was originally developed as an anticancer agent.

IFN-α and AZT induced TRAIL-mediated apoptosis of PEL [80,81]. IFN-α upregulates TRAIL in PEL cells, while AZT sensitizes them to TRAIL, thus resulting in the

activation of a suicide program. The efficacy of this approach needs to be validated in clinical trials.

2.6.10 Antibody-based therapy

Although rituximab, a chimeric anti-CD20 antibody, has provided a significant survival advantage for B-cell NHL in combination with standard chemotherapy, rituximab does not play a significant therapeutic role in PEL because CD20 is not usually expressed on the surface of PEL cells. Only rare cases expressing CD20 have been reported to respond to rituximab [82].

Brentuximab vedotin (SGN-35) is an antibody–drug conjugate in which a chimeric anti-CD30 antibody is combined with the synthetic microtubule-disrupting agent monomethyl auristatin E [83]. Recently, brentuximab vedotin was approved for the treatment of CD30-positive relapsed Hodgkin's lymphoma (HL) and systemic ALCL [84]. Treatment with brentuximab vedotin also prolonged the survival of a PEL xenograft mouse model [85]. Subsequence clinical studies in HL and ALCL patients would provide evidence for the clinical use in PEL.

PEL cells secrete vascular endothelial growth factor (VEGF)-A [86]. Moreover, treatment with mouse anti-human VEGF-A monoclonal antibody inhibited the development of ascites in a xenograft mouse model. Bevacizumab, a humanized VEGF-A monoclonal antibody, is clinically used for the treatment of a variety of human cancers, including colorectal, non-small-cell lung, ovarian and metastatic renal cell carcinomas [87]. Evaluation of the effect of bevacizumab on PEL *in vivo* is needed.

2.6.11 High-dose chemotherapy with autologous stem cell transplantation

Efficacy of high-dose chemotherapy with autologous stem cell transplantation (ASCT) for chemotherapy-sensitive relapsed disease in HIV-associated lymphoma has been reported [88]. Only two cases have been reported in PEL [89,90]. One failed to eradicate PEL, while another was successfully treated with high-dose chemotherapy with ASCT following CR 12 months post-transplantation. In a case of HIV-associated PBL, which also occurs specifically in HIV-infected patients and generally has a poor prognosis, ASCT has been successfully performed, resulting in CR [91]. It might, therefore, be considered for chemotherapy-sensitive relapsed disease and in patients who have a good performance status.

2.6.12 Allogeneic hematopoietic stem cell transplantation (HSCT)

Successful treatment with reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation (HSCT) in second remission has been reported [92]. This patient remained in CR at 31 months post-transplantation only on cART with undetectable HIV-viral loads. Allogeneic HSCT can be performed even in HIV-infected patients if we are aware of the drug interactions and opportunistic infections.

2.6.13 Radiation

PEL cells are sensitive to irradiation in culture and in a xenograft mouse model [93]. It was reported that chemotherapy-refractory PEL patients achieved remission and survived for > 12 months [94]. Although radiation would be a choice for the treatment of an extracavitary variant of PEL, especially, there is a need for optimization in several factors, including stage, bulky mass and extranodal sites.

2.6.14 Immunotherapy

In experimental studies using a xenograft mouse model, the efficacy of immunotherapy using natural killer cells and γδ T cells has been reported [95,96]. PEL occurs with an immunodeficient status; therefore, it is susceptible to immune cells. However, more clinical investigation is needed to perform immunotherapy against PEL.

3. Expert opinion

PEL is aggressive and resistant to conventional chemotherapy with a poor prognosis. There are several treatment precautions for the treatment of PEL. PEL is commonly accompanied by HIV infection; therefore, drug interactions between anticancer agents and cART should be carefully monitored. The management of opportunistic infection is also needed. Next, controlling malignant effusion is vital because it may have a mass effect on the organs and change the anticancer drug distribution throughout the body. Repeated drainage and the use of diuretics may be performed to discharge malignant effusion. Prior to treatment, suspecting PEL and performing the test to detect KSHV are essential if patients have effusion or solid lymphomas with plasma-cell differentiation. A flow chart illustrating the diagnostic work up of a PEL patient is depicted in Figure 2. We would caution against a diagnosis, especially in the case of other NHL with malignant effusion, because therapeutic approaches might be different for some NHLs. All patients should be evaluated for any signs of tumor lysis, including lactate dehydrogenase, electrolytes, creatinine, calcium, phosphorus and uric acid, because the clinical course of PEL is very aggressive. Appropriate interventions are the key for preventing and managing tumor lysis syndrome.

Despite the evidence of case reports and the results of preclinical experiments using mouse model, there is still no standard treatment in anti-PEL therapy. Among anticancer agents, the use of high-dose methotrexate should be avoided in patients with effusions. The accumulation of methotrexate may result in delayed clearance and systemic toxicity. To minimize cardiac toxicity, liposomal anthracyclines may offer the pharmacokinetic benefits of infusional doxorubicin. The safety and efficacy of substituting liposomal anthracyclines for doxorubicin in HIV-associated lymphoma has been reported [97,98]. Combination chemotherapies such as bortezomib-containing regimens might be beneficial for this aggressive lymphoma because there is no evidence of curing PEL with conventional systemic chemotherapy including

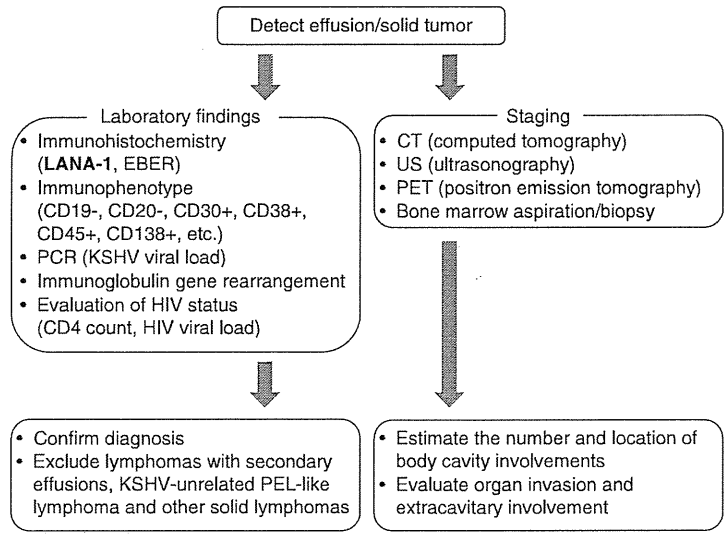

Figure 2. Flow chart illustrating diagnostic work up of a PEL patient.

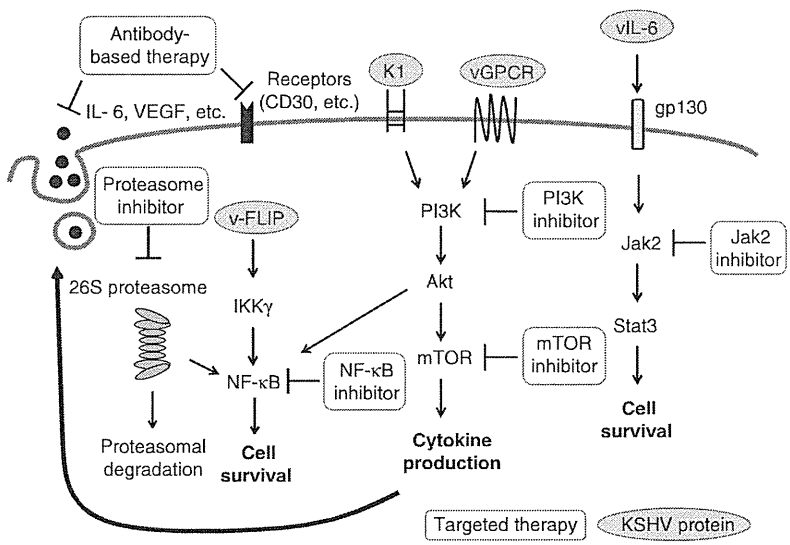

Figure 3. Potential candidate targeting molecules for the treatment of PEL are shown. PEL shows constitutive activity of signaling pathways for cytokine production and cell survival, including the NF-κB, JAK/STAT and PI3K/AKT/mTOR pathways. These signaling pathways, cytokines and surface antigens are considered for the molecular targets of PEL.

CHOP-like regimens. Moreover, Optimal treatment for relapsed or refractory PEL has not been defined. The regimens used for relapse or refractory NHL, such as ESHAP (etoposide, solumedrol, high-dose cytosine arabinoside, platinum) and ICE (ifosmide, cisplatin, etoposide), have been considered [99,100]. If patients have chemosensitive disease and a good performance status, high-dose chemotherapy with ASCT may be an option.

The implementation of cART appears to be associated with a better prognosis in trials with other HIV-associated lymphomas [101,102]. In addition, there are case reports of PEL patients achieving prolonged remission with cART alone [42,43]. Thus,

initiation or continuation of cART is recommended for the treatment of HIV-infected PEL patients.

Attention to supportive treatment for opportunistic infections is important in HIV-infected PEL patients. Granulocytes colony-stimulating factor helps reduce chemotherapy-induced neutropenic complications. All patients need to receive prophylaxis for *Pneumocystis carinii* pneumonia, regardless of the CD4 cell count. For patients who have severe neutropenia with chemotherapy, alternatives to trimethoprim-sulfamethoxazole for *Pneumocystis carinii* prophylaxis can be considered, including dapsone or aerosolized pentamidine. Infectious complications may be minimized by using prophylactic fluoroquinolone antibiotics and azoles during periods of protracted neutropenia.

It should be recognized that there is a significant risk of drug interactions between chemotherapy and cART regimens, particularly protease inhibitor-based regimens. Raltegravir-based regimens are less frequently associated with drug interactions. Close communication between the oncologist and the patient's primary HIV-treating physician is also important to avoid drug interactions in chemotherapy.

Better understanding of the oncogenesis of KSHV would lead to the development of novel therapies in PEL. Potential candidate targeting molecules for the treatment of PEL are shown in **Figure 3**. PEL displays constitutive activity of many signaling pathways in survival and growth, including the NF-κB, JAK/STAT and PI3K/AKT/mTOR pathways. These constitutive activated signal cascades are also seen in resistant hematological malignancies such as multiple myeloma; therefore, the clinical evaluation of novel agents targeting these signaling pathways in resistant hematological malignancies would provide information for their clinical use in PEL.

Declaration of interest

This study was supported in part by a Health and Labour Sciences Research Grant from the Ministry of Health, Labour, and Welfare of Japan (H25-AIDS-I-002). The authors state no conflict of interest and have received no payment for the preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Beral V, Peterman T, Berkelman R, et al. AIDS-associated non-Hodgkin lymphoma. Lancet 1991;337:805-9
2. Simonelli C, Spina M, Cinelli R, et al. Clinical features and outcome of primary effusion lymphoma in HIV-infected patients: a single-institution study. J Clin Oncol 2003;21:3948-54
3. Knowles DM, Inghirami G, Ubriaco A, et al. Molecular genetic analysis of three AIDS-associated neoplasms of uncertain lineage demonstrates their B-cell derivation and the possible pathogenetic role of the Epstein-Barr virus. Blood 1989;73:792-9
4. Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 1995;332:1186-91

•• **Kaposi's sarcoma-associated herpes virus (KSHV) DNA sequences was identified within a distinct subtype of AIDS-related lymphoma presenting with lymphomatous effusions for the first time.**

5. Nador RG, Cesarman E, Chadburn A, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma-associated herpes virus. Blood 1996;88:645-56

•• **Primary effusion lymphoma (PEL) was proposed as a distinct entity associated with KSHV.**

6. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 1994;266:1865-9
7. Soulier J, Grollet L, Oksenhendler E, et al. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 1995;86:1276-80
8. Carbone A, Cesarman E, Spina M, et al. HIV-associated lymphomas and gamma-herpesviruses. Blood 2009;113:1213-24
9. Carbone A, Cesarman E, Gloghini A, et al. Understanding pathogenetic aspects and clinical presentation of primary effusion lymphoma through its derived cell lines. AIDS 2010;24:479-90

• **Summary of characteristic features in PEL cell lines.**

10. Goto H, Kojima Y, Nagai H, et al. Establishment of a CD4-positive cell line from an AIDS-related primary effusion lymphoma. Int J Hematol 2013;97:624-33
11. Ansari MQ, Dawson DB, Nador R, et al. Primary body cavity-based AIDS-related lymphomas. Am J Clin Pathol 1996;105:221-9
12. Jones D, Ballestas ME, Kaye KM, et al. Primary-effusion lymphoma and Kaposi's sarcoma in a cardiac-transplant recipient. N Engl J Med 1998;339:444-9

▶13. Klepfish A, Sarid R, Shtalrid M, et al. Primary effusion lymphoma (PEL) in HIV-negative patients–a distinct clinical entity. Leuk Lymphoma 2001;41:439-43

14. Dukers NH, Rezza G. Human herpesvirus 8 epidemiology: what we do and do not know. AIDS 2003;17:1717-30
15. Chadburn A, Hyjek E, Mathew S, et al. KSHV-positive solid lymphomas represent an extra-cavitary variant of primary effusion lymphoma. Am J Surg Pathol 2004;28:1401-16

• **Extracavitary variant of PEL was reported.**

16. Pan L, Milligan L, Michaeli J, et al. Polymerase chain reaction detection of Kaposi's sarcoma-associated herpesvirus-optimized protocols and their application to myeloma. J Mol Diagn 2001;3:32-8
17. Drexler HG, Meyer C, Gaidano G, et al. Constitutive cytokine production by primary effusion (body cavity-based) lymphoma-derived cell lines. Leukemia 1999;13:634-40

• **Cytokine producion in PEL cell lines.**

18. Michai M, Goto H, Hattori S, et al. Soluble CD30: a possible serum tumor marker for primary effusion lymphoma. Asian Pac J Cancer Prev 2012;13:4939-41

19. Friborg J Jr, Kong W, Hottiger MO, et al. p53 inhibition by the LANA protein of KSHV protects against cell death. Nature 1999;402:889-94

20. Radkov SA, Kellam P, Boshoff C. The latent nuclear antigen of Kaposi sarcoma-associated herpesvirus targets the retinoblastoma-E2F pathway and with the oncogene Hras transforms primary rat cells. Nat Med 2000;6:1121-7

21. Swanton C, Mann DJ, Fleckenstein B, et al. Herpes viral cyclin/Cdk6 complexes evade inhibition by CDK inhibitor proteins. Nature 1997;390:184-7

22. Thome M, Schneider P, Hofmann K, et al. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 1997;386:517-21

23. Matta H, Chaudhary PM. Activation of alternative NF-kappa B pathway by human herpes virus 8-encoded Fas-associated death domain-like IL-1 beta-converting enzyme inhibitory protein (vFLIP). Proc Natl Acad Sci USA 2004;101:9399-404

•• **Activation of NF-κB pathway by vFLIP.**

24. Kliche S, Nagel W, Kremmer E, et al. Signaling by human herpesvirus 8 kaposin A through direct membrane recruitment of cytohesin-1. Mol Cell 2001;7:833-43

25. McCormick C, Ganem D. The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. Science 2005;307:739-41

26. Molden J, Chang Y, You Y, et al. A Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit. J Biol Chem 1997;272:19625-31

27. Munoz-Fontela C, Marcos-Villar L, Hernandez F, et al. Induction of paclitaxel resistance by the Kaposi's sarcoma-associated herpesvirus latent protein LANA2. J Virol 2008;82:1518-25

28. Gao SJ, Boshoff C, Jayachandra S, et al. KSHV ORF K9 (vIRF) is an oncogene which inhibits the interferon signaling pathway. Oncogene 1997;15:1979-85

29. Gottwein E, Mukherjee N, Sachse C, et al. A viral microRNA functions as an orthologue of cellular miR-155. Nature 2007;450:1096-9

30. Skalsky RL, Samols MA, Plaisance KB, et al. Kaposi's sarcoma-associated herpesvirus encodes an ortholog of miR-155. J Virol 2007;81:12836-45

31. Matolcsy A, Nador RG, Cesarman E, et al. Immunoglobulin VH gene mutational analysis suggests that primary effusion lymphomas derive from different stages of B cell maturation. Am J Pathol 1998;153:1609-14

32. Klein U, Gloghini A, Gaidano G, et al. Gene expression profile analysis of AIDS-related primary effusion lymphoma (PEL) suggests a plasmablastic derivation and identifies PEL-specific transcripts. Blood 2003;101:4115-21

33. Roy D, Sin SH, Damania B, et al. Tumor suppressor genes FHIT and WWOX are deleted in primary effusion lymphoma (PEL) cell lines. Blood 2011;118:e32-9

34. Nakatsuka S, Yao M, Hoshida Y, et al. Pyothorax-associated lymphoma: a review of 106 cases. J Clin Oncol 2002;20:4255-60

35. Xiao J, Selvaggi SM, Leith CP, et al. Kaposi sarcoma herpesvirus/human herpesvirus-8-negative effusion-based lymphoma: report of 3 cases and review of the literature. Cancer Cytopathol 2013

36. Alexanian S, Said J, Lones M, et al. KSHV/HHV8-negative effusion-based lymphoma, a distinct entity associated with fluid overload states. Am J Surg Pathol 2013;37:241-9

• **KSHV-negative effusion-based lymphoma was proposed as a distinct entity associated with fluid overload states.**

37. Chan AC, Chan JK, Yan KW, et al. Anaplastic large cell lymphoma presenting as a pleural effusion and mimicking primary effusion lymphoma. A report of 2 cases. Acta Cytol 2003;47:809-16

38. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993;329:987-94

39. Boulanger E, Gerard L, Gabarre J, et al. Prognostic factors and outcome of human herpesvirus 8-associated primary effusion lymphoma in patients with AIDS. J Clin Oncol 2005;23:4372-80

▶40. Castillo JJ, Shum H, Lahijani M, et al. Prognosis in primary effusion lymphoma is associated with the number of body cavities involved. Leuk Lymphoma 2012;53:2378-82

41. Boulanger E, Daniel MT, Agbalika F, et al. Combined chemotherapy including high-dose methotrexate in KSHV/HHV8-associated primary effusion lymphoma. Am J Hematol 2003;73:143-8

42. Oksenhendler E, Clauvel JP, Jouveshomme S, et al. Complete remission of a primary effusion lymphoma with antiretroviral therapy. Am J Hematol 1998;57:266

• **Complete remission of a PEL patient with sole combination antiretroviral therapy was reported.**

43. Hocqueloux L, Agbalika F, Oksenhendler E, et al. Long-term remission of an AIDS-related primary effusion lymphoma with antiviral therapy. AIDS 2001;15:280-2

44. Bower M, McCall-Peat N, Ryan N, et al. Protease inhibitors potentiate chemotherapy-induced neutropenia. Blood 2004;104:2943-6

45. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 2005;352:2487-98

46. O'Connor OA, Wright J, Moskowitz C, et al. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol 2005;23:676-84

47. Keller SA, Schattner EJ, Cesarman E. Inhibition of NF-kappaB induces apoptosis of KSHV-infected primary effusion lymphoma cells. Blood 2000;96:2537-42

48. Obeng EA, Carlson LM, Gutman DM, et al. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. Blood 2006;107:4907-16

49. Nikrad M, Johnson T, Puthalalath H, et al. The proteasome inhibitor bortezomib sensitizes cells to killing by death receptor ligand TRAIL via

BH3-only proteins Bik and Bim. Mol Cancer Ther 2005;4:443-9

50. Boulanger E, Meignin V, Oksenhendler E. Bortezomib (PS-341) in patients with human herpesvirus 8-associated primary effusion lymphoma. Br J Haematol 2008;141:559-61

51. An J, Sun Y, Fisher M, et al. Antitumor effects of bortezomib (PS-341) on primary effusion lymphomas. Leukemia 2004;18:1699-704

52. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5:749-59

53. Sarosiek KA, Cavallin LE, Bhatt S, et al. Efficacy of bortezomib in a direct xenograft model of primary effusion lymphoma. Proc Natl Acad Sci USA 2010;107:13069-74

54. Vaeteewoottacharn K, Kariya R, Matsuda K, et al. Perturbation of proteasome function by bortezomib leading to ER stress-induced apoptotic cell death in cholangiocarcinoma. J Cancer Res Clin Oncol 2013;139:1551-62

55. Dabaghmanesh N, Matsubara A, Miyake A, et al. Transient inhibition of NF-kappaB by DHMEQ induces cell death of primary effusion lymphoma without HHV-8 reactivation. Cancer Sci 2009;100:737-46

56. Takahashi-Makise N, Suzu S, Hiyoshi M, et al. Biscoclaurine alkaloid cepharanthine inhibits the growth of primary effusion lymphoma in vitro and in vivo and induces apoptosis via suppression of the NF-kappaB pathway. Int J Cancer 2009;125:1464-72

57. Matsuno T, Kariya R, Yano S, et al. Diethyldithiocarbamate induces apoptosis in HHV-8-infected primary effusion lymphoma cells via inhibition of the NF-kappaB pathway. Int J Oncol 2012;40:1071-8

58. Goto H, Kariya R, Shimamoto M, et al. Antitumor effect of berberine against primary effusion lymphoma via inhibition of NF-kappaB pathway. Cancer Sci 2012;103:775-81

59. Gopalakrishnan R, Matta H, Chaudhary PM. A purine scaffold HSP90 inhibitor BIIB021 has selective activity against KSHV-associated Primary Effusion Lymphoma and blocks vFLIP K13-induced NF-kappaB. Clin Cancer Res 2013

60. Nayar U, Lu P, Goldstein RL, et al. Targeting the Hsp90-associated viral oncoproteome in gammaherpesvirus-associated malignancies. Blood 2013

61. Aoki Y, Feldman GM, Tosato G. Inhibition of STAT3 signaling induces apoptosis and decreases survivin expression in primary effusion lymphoma. Blood 2003;101:1535-42

62. Reed JC. The Survivin saga goes in vivo. J Clin Invest 2001;108:965-9

63. Uddin S, Hussain AR, Manogaran PS, et al. Curcumin suppresses growth and induces apoptosis in primary effusion lymphoma. Oncogene 2005;24:7022-30

64. Uddin S, Hussain AR, Al-Hussein KA, et al. Inhibition of phosphatidylinositol 3'-kinase/AKT signaling promotes apoptosis of primary effusion lymphoma cells. Clin Cancer Res 2005;11:3102-8

• **Constitutive activation of PI3K/AKT pathway and efficacy of inhibiting PI3K/AKT in PEL cell lines.**

65. Tomlinson CC, Damania B. The K1 protein of Kaposi's sarcoma-associated herpesvirus activates the Akt signaling pathway. J Virol 2004;78:1918-27

66. Wang L, Dittmer DP, Tomlinson CC, et al. Immortalization of primary endothelial cells by the K1 protein of Kaposi's sarcoma-associated herpesvirus. Cancer Res 2006;66:3658-66

67. Boulanger E, Marchio A, Hong SS, et al. Mutational analysis of TP53, PTEN, PIK3CA and CTNNB1/beta-catenin genes in human herpesvirus 8-associated primary effusion lymphoma. Haematologica 2009;94:1170-4

• **Mutational analysis of TP53, PTEN, PIK3CA and CTNNB1/β-catenin genes in PEL cells.**

68. Hussain AR, Ahmed M, Ahmed S, et al. Thymoquinone suppresses growth and induces apoptosis via generation of reactive oxygen species in primary effusion lymphoma. Free Radic Biol Med 2011;50:978-87

69. Hussain AR, Ahmed SO, Ahmed M, et al. Cross-talk between NFkB and the PI3-kinase/AKT pathway can be targeted in primary effusion lymphoma (PEL) cell lines for efficient apoptosis. PLoS One 2012;7:e39945

70. Sin SH, Roy D, Wang L, et al. Rapamycin is efficacious against primary effusion lymphoma (PEL) cell lines in vivo by inhibiting autocrine signaling. Blood 2007;109:2165-73

• **Efficacy of mTOR inhibitor rapamycin *in vitro* and *in vivo*.**

71. Boulanger E, Afonso PV, Yahiaoui Y, et al. Human herpesvirus-8 (HHV-8)-associated primary effusion lymphoma in two renal transplant recipients receiving rapamycin. Am J Transplant 2008;8:707-10

72. Bayascas JR, Alessi DR. Regulation of Akt/PKB Ser473 phosphorylation. Mol Cell 2005;18:143-5

73. Sarbassov DD, Guertin DA, Ali SM, et al. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005;307:1098-101

74. Luppi M, Trovato R, Barozzi P, et al. Treatment of herpesvirus associated primary effusion lymphoma with intracavity cidofovir. Leukemia 2005;19:473-6

75. Halfdanarson TR, Markovic SN, Kalokhe U, et al. A non-chemotherapy treatment of a primary effusion lymphoma: durable remission after intracavitary cidofovir in HIV negative PEL refractory to chemotherapy. Ann Oncol 2006;17:1849-50

76. Kedes DH, Ganem D. Sensitivity of Kaposi's sarcoma-associated herpesvirus replication to antiviral drugs. Implications for potential therapy. J Clin Invest 1997;99:2082-6

77. Klass CM, Krug LT, Pozharskaya VP, et al. The targeting of primary effusion lymphoma cells for apoptosis by inducing lytic replication of human herpesvirus 8 while blocking virus production. Blood 2005;105:4028-34

78. Fanger NA, Maliszewski CR, Schooley K, et al. Human dendritic cells mediate cellular apoptosis via tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). J Exp Med 1999;190:1155-64

79. Griffith TS, Wiley SR, Kubin MZ, et al. Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL. J Exp Med 1999;189:1343-54

80. Toomey NL, Deyev VV, Wood C, et al. Induction of a TRAIL-mediated suicide program by interferon alpha in primary

effusion lymphoma. Oncogene 2001;20:7029-40

81. Wu W, Rochford R, Toomey L, et al. Inhibition of HHV-8/KSHV infected primary effusion lymphomas in NOD/SCID mice by azidothymidine and interferon-alpha. Leuk Res 2005;29:545-55

82. Lim ST, Rubin N, Said J, et al. Primary effusion lymphoma: successful treatment with highly active antiretroviral therapy and rituximab. Ann Hematol 2005;84:551-2

83. Francisco JA, Cerveny CG, Meyer DL, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. Blood 2003;102:1458-65

84. de Claro RA, McGinn K, Kwitkowski V, et al. U.S. Food and Drug Administration approval summary: brentuximab vedotin for the treatment of relapsed Hodgkin lymphoma or relapsed systemic anaplastic large-cell lymphoma. Clin Cancer Res 2012;18:5845-9

85. Bhatt S, Ashlock BM, Natkunam Y, et al. CD30 targeting with brentuximab vedotin: a novel therapeutic approach to primary effusion lymphoma. Blood 2013;122:1233-42

86. Aoki Y, Tosato G. Role of vascular endothelial growth factor/vascular permeability factor in the pathogenesis of Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphomas. Blood 1999;94:4247-54

• **VEGF stimulation of vascular permeability is critical to the pathogenesis of PEL.**

87. Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. Clin Ther 2006;28:1779-802

88. Re A, Cattaneo C, Michieli M, et al. High-dose therapy and autologous peripheral-blood stem-cell transplantation as salvage treatment for HIV-associated lymphoma in patients receiving highly active antiretroviral therapy. J Clin Oncol 2003;21:4423-7

89. Waddington TW, Aboulafia DM. Failure to eradicate AIDS-associated primary effusion lymphoma with high-dose chemotherapy and autologous stem cell reinfusion: case report and literature review. AIDS Patient Care STDS 2004;18:67-73

90. Won JH, Han SH, Bae SB, et al. Successful eradication of relapsed primary effusion lymphoma with high-dose chemotherapy and autologous stem cell transplantation in a patient seronegative for human immunodeficiency virus. Int J Hematol 2006;83:328-30

91. Goto H, Hagiwara S, Hirai R, et al. Case of relapsed AIDS-related plasmablastic lymphoma treated with autologous stem cell transplantation and highly active antiretroviral therapy. Rare Tumors 2011;3:e11

92. Bryant A, Milliken S. Successful reduced-intensity conditioning allogeneic HSCT for HIV-related primary effusion lymphoma. Biol Blood Marrow Transplant 2008;14:601-2

93. Shiraishi Y, Gotoh K, Towata T, et al. Therapeutic effects of gamma-irradiation in a primary effusion lymphoma mouse model. Exp Ther Med 2010;79-84

94. Cassoni A, Ali U, Cave J, et al. Remission after radiotherapy for a patient with chemotherapy-refractory HIV-associated primary effusion lymphoma. J Clin Oncol 2008;26:5297-9

95. Dewan MZ, Terunuma H, Toi M, et al. Potential role of natural killer cells in controlling growth and infiltration of AIDS-associated primary effusion lymphoma cells. Cancer Sci 2006;97:1381-7

96. Goto H, Matsuda K, Srikoon P, et al. Potent antitumor activity of zoledronic acid-induced Vgamma9Vdelta2 T cells against primary effusion lymphoma. Cancer Lett 2013;331:174-82

97. Levine AM, Tulpule A, Espina B, et al. Liposome-encapsulated doxorubicin in combination with standard agents (cyclophosphamide, vincristine, prednisone) in patients with newly diagnosed AIDS-related non-Hodgkin's lymphoma: results of therapy and correlates of response. J Clin Oncol 2004;22:2662-70

98. Combs S, Neil N, Aboulafia DM. Liposomal doxorubicin, cyclophosphamide, and etoposide and antiretroviral therapy for patients with AIDS-related lymphoma: a pilot study. Oncologist 2006;11:666-73

99. Bi J, Espina BM, Tulpule A, et al. High-dose cytosine-arabinoside and cisplatin regimens as salvage therapy for refractory or relapsed AIDS-related non-Hodgkin's lymphoma. J Acquir Immune Defic Syndr 2001;28:416-21

100. Kewalramani T, Zelenetz AD, Nimer SD, et al. Rituximab and ICE as second-line therapy before autologous stem cell transplantation for relapsed or primary refractory diffuse large B-cell lymphoma. Blood 2004;103:3684-8

101. Vaccher E, Spina M, Talamini R, et al. Improvement of systemic human immunodeficiency virus-related non-Hodgkin lymphoma outcome in the era of highly active antiretroviral therapy. Clin Infect Dis 2003;37:1556-64

102. Lim ST, Karim R, Nathwani BN, et al. AIDS-related Burkitt's lymphoma versus diffuse large-cell lymphoma in the pre-highly active antiretroviral therapy (HAART) and HAART eras: significant differences in survival with standard chemotherapy. J Clin Oncol 2005;23:4430-8

Affiliation

Hiroki Goto & Seiji Okada†
†Author for correspondence
Kumamoto University, Center for AIDS Research, Division of Hematopoiesis, 2-2-1, Honjo, Chuo-ku, Kumamoto, 860-0811, Japan
Tel: +81 96 373 6522;
Fax: +81 96 373 6523;
E-mail: okadas@kumamoto-u.ac.jp

Cancer Letters 342 (2014) 52–59

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

HIV protease inhibitor Lopinavir induces apoptosis of primary effusion lymphoma cells via suppression of NF-κB pathway

Ryusho Kariya [a], Manabu Taura [a], Shinya Suzu [a], Hirofumi Kai [b], Harutaka Katano [c], Seiji Okada [a,*]

[a] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, 2-2-1, Honjo, Kumamoto 860-0811, Japan
[b] Department of Molecular Medicine, Graduate School of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-Honmachi, Kumamoto 862-0973, Japan
[c] Department of Pathology, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku, Tokyo 162-8640, Japan

ARTICLE INFO

Article history:
Received 29 May 2013
Received in revised form 1 August 2013
Accepted 19 August 2013

Keywords:
Primary effusion lymphoma
HIV-1 protease inhibiton
NF-κB
IκB kinase

ABSTRACT

Primary effusion lymphoma (PEL) is a non-Hodgkin lymphoma that occurs predominantly in patients with advanced AIDS. In this study, we examined the effect of HIV protease inhibitors, Lopinavir (LPV), Ritonavir (RTV) and Darunavir (DRV) on PEL cell lines *in vitro* and *in vivo*. LPV and RTV, but not DRV induced caspase-dependent apoptosis and suppressed NF-κB activity by inhibiting IKK phosphorylation in PEL cells. In a PEL xenograft mouse model, LPV significantly inhibited the growth and invasion of PEL cells. These results suggest that LPV may have promise for the treatment and prevention of PEL, which occurs in HIV/AIDS patients.

1. Introduction

Primary effusion lymphoma (PEL) is a subtype of non-Hodgkin B cell lymphoma that mainly presents in patients with advanced AIDS, but is sometimes also found in immunosuppressed patients, such as those who have undergo organ transplantation [1,2]. Among AIDS-related NHLs, PEL generally has an extremely aggressive clinical course with a median survival of only 6 months [2,3]. PEL usually presents as a lymphomatous effusion in body cavities and is caused by Kaposi sarcoma-associated herpes virus (KSVH/HHV-8) [1]. A number of constitutively activated signaling pathways play critical roles in the survival and growth of PEL cells. These include nuclear factor (NF)-κB, JAK/STAT and PI3 kinase [4–6]. KSHV/HHV-8 encodes a virus Fas-associated death domain-like interleukin-1β-converting enzyme (FLICE) inhibitory protein (vFLIP) that has the ability to activate the NF-κB pathway [7–9]. vFLIP has been shown to bind to the IKK complex to induce constitutive kinase activation [10] and, as a result, PEL cells have high levels of nuclear NF-κB activity, whereas inhibition of NF-κB induces apoptosis in PEL cells [5,11]. These studies support the idea that vFLIP-mediated NF-κB activation is necessary for the survival of PEL cells and that this pathway represents a target for molecular therapy for this disease.

HIV-1 protease inhibitors (HIV-PIs) have been successfully used in the treatment of HIV-1 infection. Incorporation of HIV-PIs in combination antiretroviral therapy (cART) has significantly reduced morbidity and mortality and prolonged the lifespan of patients with HIV infection. However, HIV-PIs have been shown to directly affect cell metabolism, interfere with host proteases and induce metabolic abnormalities such as insulin resistance, lipodystrophy, and hyperlipidemia, even though they were designed to selectively interfere with the catalytic site of HIV protease. Recently, HIV-PIs have become a focus of attention for having anti-tumor effects [12]. HIV-PIs have been shown to block angiogenesis, tumor cell invasion and tumor cell growth, and to induce endothelial reticulum stress, autophagy and tumor cell apoptosis both *in vivo* and *in vitro* [13–15]. Interestingly, the mechanisms of these anti-tumor effects are different with each HIV-PI, indicating that, although classified together, HIV-PIs are quite distinct compounds [16].

Ritonavir (RTV) has been shown to inhibit the chymotrypsin-like activity of the 20S proteasome and to activate the chymotrypsin-like activity of the 26S proteasome conversely [17–19]. RTV also has been reported to inhibit the transactivation of NF-κB induced by activators such as TNFα, HIV-1 Tat protein and the human herpesvirus 8 protein ORF74 [20]. It is possible that inhibition of NF-κB activation by RTV is linked to additional pathways other than proteasome inhibition. HIV-PIs also have been shown to have direct antiangiogenic and antitumor activity [12]. Recently, it was reported that RTV inhibits the growth and infiltration of ATL cells through targeting NF-κB [14,21]. Lopinavir (LPV) is a frequently used HIV-PI, but only a few antitumor effects have been reported [22]. Recently, a second generation HIV-PI,

* Corresponding author. Tel.: +81 96 373 6522; fax: +81 96 373 6523.
E-mail address: okadas@kumamoto-u.ac.jp (S. Okada).

0304-3835/$ - see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.canlet.2013.08.045