blood®
Review Article

Biology and management of primary effusion lymphoma

Kazuyuki Shimada, Fumihiko Hayakawa, and Hitoshi Kiyoi

Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan

Primary effusion lymphoma (PEL) is a rare B-cell malignancy that most often occurs in immunocompromised patients, such as HIV-infected individuals and patients receiving organ transplantation. The main characteristic of PEL is neoplastic effusions in body cavities without detectable tumor masses. The onset of the disease is associated with latent infection of human herpes virus 8/Kaposi sarcoma–associated herpes virus, and the normal counterpart of tumor cells is B cells with plasmablastic differentiation. A condition of immunodeficiency and a usual absence of CD20 expression lead to the expectation of the lack of efficacy of anti-CD20 monoclonal antibody; clinical outcomes of the disease remain extremely poor, with an overall survival at 1 year of ~30%. Although recent progress in antiretroviral therapy has improved outcomes of HIV-infected patients, its benefit is still limited in patients with PEL. Furthermore, the usual high expression of programmed death ligand 1 in tumor cells, one of the most important immune-checkpoint molecules, results in the immune escape of tumor cells from the host immune defense, which could be the underlying mechanism of poor treatment efficacy. Molecular-targeted therapies for the activating pathways in PEL, including NF-κB, JAK/STAT, and phosphatidylinositol 3-kinase/AKT, have emerged to treat this intractable disease. A combination of immunological recovery from immune deficiency, overcoming the immune escape, and the development of more effective drugs will be vital for improving the outcomes of PEL patients in the future. (*Blood*. 2018;132(18):1879-1888)

Introduction

Primary effusion lymphoma (PEL) is a rare distinct disease entity of large B-cell lymphoma according to the current World Health Organization (WHO) classification.[1] PEL is characterized by the presence of significant neoplastic effusions in the body cavity without detectable tumor masses. The disease most often occurs in immunocompromised patients, such as HIV-infected individuals and patients receiving organ transplantation; the onset of the disease is associated with infection by human herpes virus 8 (HHV8)/Kaposi sarcoma (KS)–associated herpes virus (KSHV). Related to the setting of immunodeficiency, the prognosis of PEL has been poor even with attempts to recover the host immune defense by the use of antiretroviral therapies (ARTs) and to develop molecular-targeted therapies against the activated tumor proliferation and/or survival signaling. In this review, the cutting edge of the biology and the therapeutic strategies for this intractable lymphoma are described and discussed.

History and definition of PEL

PEL was first described in 1989 as an AIDS-related lymphoma in which patients with the disease developed further weakness and cachexia with a large malignant pleural effusion.[2] In 1995, Cesarman et al identified KSHV in tumor cells of AIDS-related lymphoma with malignant effusion,[3] and Nador et al called HHV8-associated lymphoma whose main tumor was present in the body cavity fluid PEL in 1996.[4] As mentioned in the Introduction, PEL is defined as a large B-cell neoplasm usually presenting as serous effusions without detectable tumor masses in the WHO classification.[1] The disease has a subtype defined as HHV8/KSHV$^+$ tumor mass formation with morphologic and phenotypic characteristics similar to those of PEL in extranodal sites, namely extracavitary PEL.[5-7]

Morphology and immunophenotype

PEL is typically diagnosed on the basis of cytological preparation of effusion fluid. PEL cells exhibit marked variation in size, ranging from large immunoblastic or plasmablastic cells to cells with more anaplastic morphology (Figure 1).[1] The nuclei of these cells are large and round to more irregular in shape, with prominent nucleoli. The cytoplasm can be abundant and is deeply basophilic with vacuoles in occasional cells. Poorly defined perinuclear hofs are often observed, and anaplastic cells that resemble Reed-Sternberg cells in classical Hodgkin lymphoma may be detected. The histological features of extracavitary PEL are similar to those seen in effusions. Tingible body macrophages and numerous mitotic figures with or without a starry-sky appearance are seen in tissue sections. The marked pleomorphism characteristically seen in cytological specimens may not be apparent in tissue sections.[7,8]

The lymphoma cells usually express CD45, but they lack pan–B-cell antigens, including CD19, CD20, and CD79a (Figure 1).[2,4] Surface and cytoplasmic immunoglobulins are usually negative.

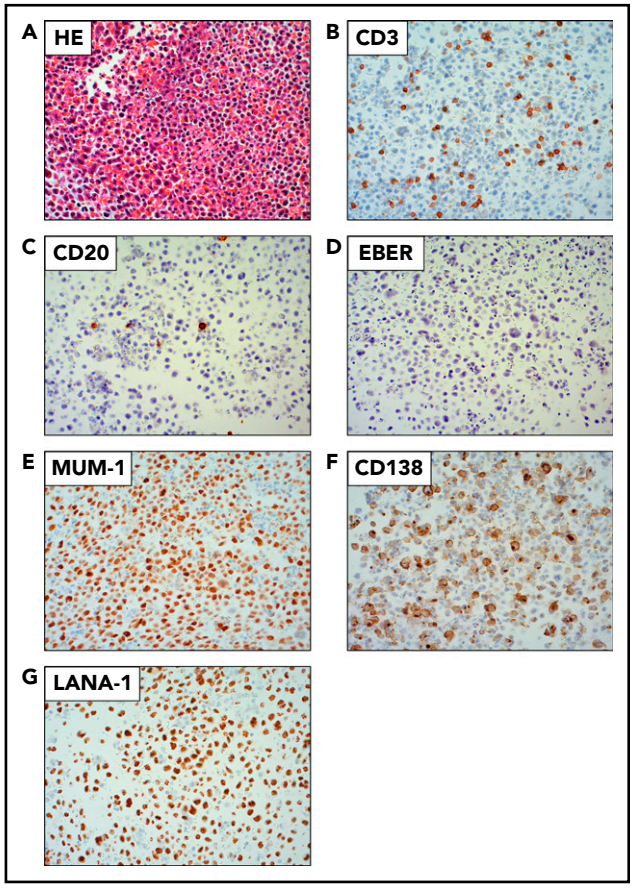

Figure 1. Pathological specimens of PEL. Specimens of a cell block from an HIV$^-$ elderly patient with PEL stained by hematoxylin and eosin (HE; A), CD3 (B), CD20 (C), EBV-encoded small RNA (EBER; D), multiple myeloma oncogene 1 (MUM-1; E), CD138 (F), and LANA-1 (G) are shown (original magnification ×200). Staining of CD3 and CD20 on immunohistochemistry (IHC) and EBER in situ hybridization are negative, with positive staining for MUM-1, CD138, and LANA-1 on IHC. All images are courtesy of Koichi Oshima, Kurume University, Kurume, Japan.

In association with the tumor cells with plasmablastic differentiation, the lymphoma cells express plasma cell–related markers such as CD38, CD138, and VS38c.[1] In case series involving 61 patients with PEL, CD45 and CD30 were positive in 93% and 73% of patients, respectively.[9] The cells usually lack T-cell/natural killer (NK)-cell antigens, although aberrant expression of T-cell antigens may occur, especially in extracavitary PEL.[5,10] The nuclei of the tumor cells are positive for HHV8/KSHV-associated protein latency-associated nuclear antigen 1 (LANA-1), which is useful for the diagnosis of PEL.[11] The normal counterpart of tumor cells is post–germinal center B cells demonstrating plasmablastic differentiation.[1] In association with plasmablastic differentiation, plasmablastic lymphoma (PBL) must be considered in the differential diagnosis of PEL.[12] The morphology of PBL cells is similar to that of PEL cells; tumor cells can be immunoblastic with prominent central nucleoli or plasmablastic with abundant basophilic cytoplasm. PBL displays an immunophenotype that closely resembles that of PEL, with expression of plasma cell–related markers, absence of pan–B-cell antigens, and frequent positivity for Epstein-Barr virus (EBV). However, PBL can be distinguished from extracavitary PEL by its universal association with HHV8/KSHV infection.[13]

Biological features

HHV8/KSHV infection

HHV8/KSHV is a vital factor in developing PEL and has double-stranded DNA. The viral DNA consists of 145 kb of a unique genome sequence termed the long unique region (LUR) with long terminal repeat sequences of 801 bp at both ends. LUR consists of about 90 open reading frames (ORFs) involved in not only genes associated with DNA synthesis, replication, and structure genes indispensable for viral replication, but also human homolog genes for proliferation, apoptosis, and cytokine signals.[14-16] Each ORF is given a number, such as ORF# or K#, where K# indicates HHV8-specific genes. Similar to other herpes viruses, HHV8/KSHV infects the host cells as 2 different forms: latent infection and lytic infection.[14] Most infected cells express a latent infection pattern regarding gene expression, whereas a very small percentage of tumor cells demonstrate gene expression of the lytic phase. Five latent gene products are thought to play a significant role in the development of PEL and are involved in lymphomagenesis: LANA-1; LANA-2/viral interferon regulatory factor-3 (vIRF-3); viral homolog of cyclin D (v-Cyclin); viral homolog of FLICE-inhibitory protein (v-FLIP); and Kaposin (K12) (Figure 2).[17] LANA-1, also called ORF73, is the most important of the latent proteins, and is useful in establishing a diagnosis of HHV8/KSHV infection. LANA-1 holds the HHV8/KSHV episome to the nuclear chromosome and replicates HHV8/KSHV DNA at mitosis, leading to its distribution to daughter cells.[18] In terms of oncogenesis associated with LANA-1, the C terminus of LANA-1 binds to TP53, resulting in the inhibition of TP53-dependent apoptosis, and it also binds to retinoblastoma (RB), inducing cell proliferation through the Rb-E2F pathway.[19,20] In addition, v-Cyclin binds to cyclin-dependent kinase 6 (CDK6), leading to the inactivation of RB protein, while v-FLIP inhibits apoptosis by blockade of caspase activation mediated by FAS and tumor necrosis factor (TNF), and it activates the NF-κB pathway through activation of IκB kinase γ (IKKγ).[21,22] Kaposin, encoded by K12, has at least 3 isoforms, termed Kaposin A, B, and C, which differ in the start site of each translation on messenger RNA (mRNA).[23] Kaposin A is involved in oncogenesis through cytokesin-1.[24] Kaposin B stabilizes cytokine expressions such as interleukin 6 (IL6) and granulocyte macrophage–colony-stimulating factor by stabilization of cytokine mRNA containing adenylate-uridylate–rich elements important for latent infection of HHV8/KSHV.[24] LANA-2/vIRF-3 is involved in drug resistance by binding to polymerized microtubules, decreasing their stability.[25]

EBV infection

With the background of immune deficiency in patients with PEL, most of the neoplastic cells are coinfected with HHV8/KSHV and EBV.[26] Similar to Burkitt lymphoma, PEL tumor cells fail to express the EBV-transforming protein EBV nuclear antigen 2 (EBNA-2) and latent membrane protein 1, while they express EBNA-1, denoted as latency I phenotype. A previous study showed that EBNA-1 displays a high degree of heterogeneity in different PEL patients; no specific EBV genotype preferentially associates with PEL and individual PEL cases consistently harbor a single EBV strain.[27] The precise pathogenic role of EBV coinfection for the development of PEL is not fully understood.[26-28]

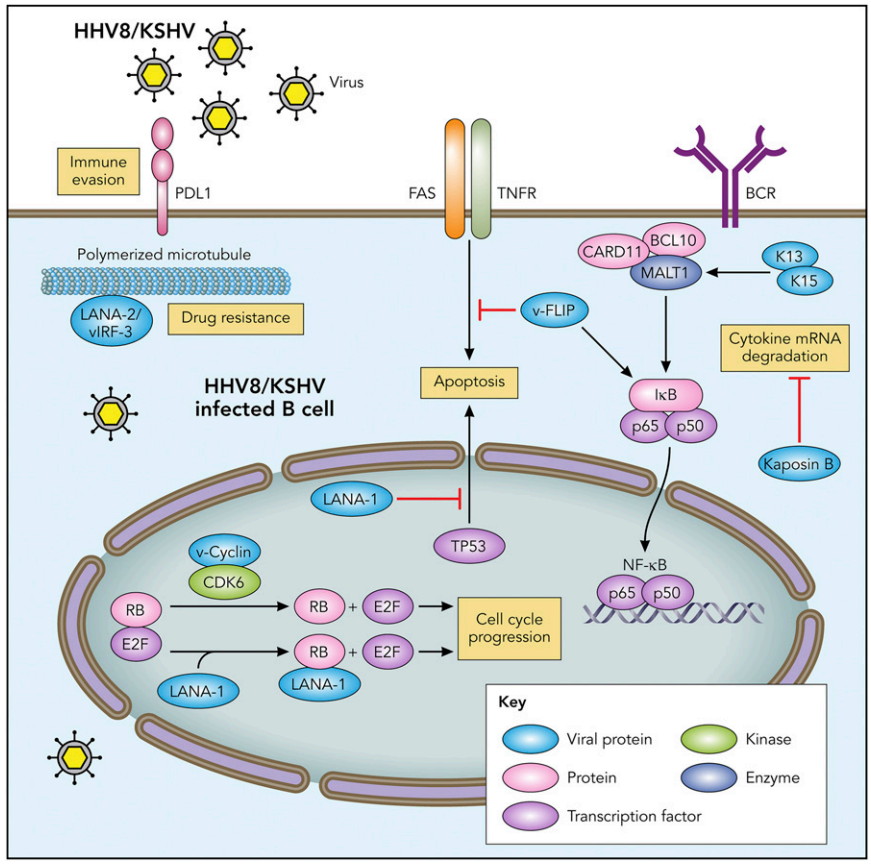

Figure 2. The schema of the proposed mechanism of PEL development. HHV8/KSHV-infected B cells acquire the characteristics of neoplastic change, such as immune evasion, drug resistance, antiapoptosis, cell cycle progression, and cytokine stabilization. Color coding is as follows: viral proteins (light blue), transcription factors (lavender), kinase (light green), enzyme (blue), and proteins (magenta). BCL10, B-cell lymphoma/leukemia 10; BCR, B-cell receptor; CARD11, caspase recruitment domain–containing protein 11; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; TNFR, TNF receptor. Professional illustration by Patrick Lane, ScEYEnce Studios.

Immune escape in PEL

Emerging findings indicate that immune evasion from host immune defense is deeply involved in the pathogenesis of malignant lymphoma.[29,30] The association of HHV8/KSHV infection with programmed death ligand 1 (PDL1) expression, known to be an immunoinhibitory molecule, has been reported. Chen et al reported PDL1 positivity, with ≥5% malignant cells in 2 of 4 patients (50%) with PEL and PDL1 positivity with ≥20% total cellularity in 3 of 4 PEL patients (75%), which indicates the potential role of immune escape in the development of PEL.[31] In fact, PDL1 is overexpressed in monocytes infected with HHV8/KSHV, leading to the production of cytokines involved in the development of KS, such as interferon 1α (IFN1α), -1β, and -6.[32] Furthermore, vIRF3 is also associated with immune escape from the host defense system in PEL.[33]

Cytogenetic features

Gene-expression profiling

Several analyses using cell lines and patient samples from EBV$^+$ and EBV$^-$ PEL provided gene-expression profiling (GEP) of PEL.[34-36] Jenner et al reported that PEL cells can be defined by the overexpression of genes involved in inflammation, cell adhesion, and invasion, which may be responsible for their presentation in body cavities.[35] Klein et al used GEP analysis of about 12 000 genes to investigate the differences among PEL, normal B cells, non-Hodgkin lymphoma (NHL) of immunocompetent hosts, and AIDS-associated NHL. The GEP of PEL was characterized as having the common features of plasmablasts and immunoblasts associated with plasmablastic differentiation of tumor cells clearly distinct from all NHLs of immunocompetent hosts and AIDS-associated NHL. Aquaporin-3, a water channel protein involved in water transport, P-selectin glycoprotein ligand 1/SELPLG, a ligand for P-selectin involved in leukocyte adhesion, and mucin1, tumor-associated glycoprotein, which were not previously associated with PEL, were identified as specific genes overexpressed in PEL.[35] Punj et al demonstrated that v-FLIP/K13, an HHV8/KSHV-encoded latent protein, upregulated the expression of a number of NF-κB–responsive genes involved in cytokine signaling, cell death, adhesion, inflammation, and immune response on gene-array analysis.[37] A comparative study of microRNA (miRNA) between HHV8/KSHV-infected cells and its associated cancers including KS and PEL demonstrated that multiple tumor suppressor miRNAs (miR-155, miR-220/221, let-7 family) were downregulated.[38]

Proteomics

A comparison of effusion and solid lymphomas in the peritoneal cavity of severe combined immunodeficiency mice resulting from the inoculation of PEL tumor cells into the peritoneal cavity has been reported. Proteomics using 2-dimensional difference gel electrophoresis and DNA microarray analyses identified 14 proteins and 105 genes, respectively, whose expressions differed significantly between effusion and solid lymphomas. Lymphocyte function-associated antigen 1, an important adhesion molecule in leukocytes, and coronin 1A, an actin-binding protein, were identified as molecules showing high expression in solid lymphoma. In addition, structural proteins (collagens, proteoglycans), adhesion molecules (integrins), and cell cycle–associated genes (MAPKs, IRF1, etc.) were also identified as

high-expression molecules in solid lymphomas. The molecules showing predominant expression in effusion lymphoma contained transactivator/cell cycle–associated genes (MAPKAPK2, C/EBPD, G protein–coupled receptor, RRAS, etc), enzymes, and a cell surface antigen (CD68).[39] The analysis of secreted proteins from PEL cell lines demonstrated proteins associated with inflammation/immune response, growth/cell cycle, mRNA processing, and structural/matrix protein; proteins with enzymatic activity were enriched in PEL.[40]

Genomics

Because of its rarity, the landscape of genomic abnormalities in PEL is largely unknown. X-chromosome–targeted sequencing identified 34 common missense mutations in 100% of PEL cases, including a Phe196Ser change in interleukin 1 receptor–associated kinase 1 (IRAK1).[41] IRAK1, modulating Toll-like receptor (TLR) signaling–mediated immune signaling, was constitutively phosphorylated and required for survival in PEL tumor cells, which implied that IRAK1 and TLR signaling were essential for a driver pathway in PEL.[41] Recent comparative analyses of PEL cell lines with other B-cell lymphoma cell lines showed that PEL cell lines had high expressions of APOBEC3B and APOBEC3C and the gene-expression signature of APOBEC family activation. Although this finding might indicate an association between expressions of APOBEC3B and APOBEC3C and HHV8/KSHV infection, the same findings were not observed in HHV8-infected cells, which indicates the potential role of the APOBEC family in PEL.[42]

Clinical features

PEL is clinically characterized by neoplastic effusions in body cavities without extracavitary tumor masses, and the most common sites are the pleural, peritoneal, and pericardial cavities. Typically, only a single cavity is involved. Patients develop dyspnea and abdominal distension as initial symptoms due to body cavity fluid. PEL usually occurs in HIV-infected individuals with a decreased number of $CD4^+$ T cells at diagnosis and accounts for 1% to 4% of HIV-related lymphomas.[43,44] PEL tumor cells are usually accompanied by coinfection with EBV,[26] and the disease has been reported in recipients of solid-organ transplants and in HHV8/KSHV-seropositive elderly patients without immunodeficiency.[45-47] The median age at diagnosis is 42 years in HIV-infected patients and 73 years in the general population without HIV infection. Male homosexual contact is the most common risk factor, followed by drug use.[1] Approximately one-third to one-half of the patients have a history of KS, and some patients have associated multicentric Castleman disease.[4,43,48] PEL without EBV coinfection usually occurs in HIV^- elderly individuals, particularly in the Mediterranean region.[49]

The disease remains restricted to the body cavities; however, some patients develop tumors in extracavitary sites including the intestinal tract, skin, lung, central nervous system, and lymph nodes, namely extracavitary PEL.[5-7] Meanwhile, PEL should be distinguished from $HHV8^-$ effusion-based lymphoma, which is morphologically similar to PEL, called PEL-like lymphoma,[50,51] and should also be distinguished from pyothorax-associated lymphoma and DLBCL with chronic inflammation, whose pathogenesis is associated with sustained inflammation.[52,53] Recently, a patient with congenital immune deficiency who developed EBV^+ and $HHV8^-$ PEL-like lymphoma has been reported, indicating that the disease can occur not only in adults, but also in children.[54]

Treatment and management

Clinical outcomes of patient series

Several reports of the clinical outcomes of PEL are summarized in Table 1. Boulanger et al reported 2 patient series.[55,56] According to the largest series involving 28 PEL patients reported by them, the median overall survival (OS) was 6.2 months, and the 1-year survival rate was 39.3%, with a median follow-up duration of 3.8 years.[55] The complete response (CR) rate with the cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP)-like regimen was 10% (1 of 10), whereas CR was achieved by other intensified regimens such as CHOP with high-dose methotrexate (MTX) and doxorubicin, cyclophosphamide, vincristine, bleomycin, and prednisone (ACVBP) in 7 of 10 patients (70%) and 1 of 2 patients, respectively.[55] Another series of 51 patients with PEL and extracavitary PEL from a single institute has recently been reported. In that report, 13 patients (26%) received a CHOP-like regimen, and 32 patients (63%) received a CHOP-like regimen with MTX. With a median follow-up of 10 years, median OS was only 10.2 months. Although the 5-year OS rates of patients receiving and not receiving MTX were 45.7% and 34.4%, respectively, the difference was not significant ($P = .72$).[57] Based on the clinical outcomes previously reported, more intensified regimens compared with the CHOP regimen might be effective in PEL patients. Although many patients infected with HIV, and even many elderly patients, are receiving intensified regimens with excellent outcomes, outcomes of patients with advanced HIV/AIDS previous to their lymphoma diagnosis and with no expectation for immune recovery are still unsatisfactory. Moreover, CD20 expression is usually absent in PEL tumor cells, which indicates the lack of effectiveness of anti-CD20 monoclonal antibody,[2,4] the key drug responsible for the recent improvement of clinical outcomes in B-cell malignancies. These characteristics are significant obstacles in the struggle against PEL. Involvement of body cavity fluid is a main characteristic of PEL, which makes administration of MTX difficult, resulting in the limited treatment choice.[58] In addition, because only a small number of studies of successful treatment with high-dose chemotherapy and autologous stem cell support (HDT/ASCT) have been reported, HDT/ASCT could be one of the treatment choices for younger patients with relapsed or refractory PEL.[59,60] However, consolidation with HDT/ASCT at the first complete remission has not been substantiated after the initial series of treatments. A limited number of patients receiving allogeneic stem cell transplantation (SCT) has also been reported.[61] In that report, a PEL patient with HIV infection received HDT/ASCT followed by reduced-intensity conditioning (RIC) allogeneic SCT for his refractory disease. His clinical course of SCT was successful without severe complications, and he survived 31 months posttransplantation only on ART, with HIV viral load remaining undetectable. Although the number of reports remains limited, RIC allogeneic SCT might be 1 treatment option.

Role of ART

ART and chemotherapies are essential to improve the clinical outcomes of PEL. Prior to the ART era, clinical outcomes of PEL were extremely unfavorable even for patients who received

Table 1. Clinical series of PEL and extracavitary PEL

	El-Fattah[71]	Olszewski et al[44]	Guillet et al[57]	Boulanger et al[55]	Simonelli et al[43]	Chadburn et al[7]	Carbone et al[6]
No. of patients	105	106	51	28	11	8	4
Disease, n							
PEL	105	106	34	28	11	—	—
Extracavitary PEL	—	—	17	—	—	8	4
Median age, y	41	44	45	44	41	40	40
Male, %	90	>95	92	96	90	100	75
HIV^+, %	NA	100	100	100	100	100	75
EBV coinfection, %	NA	NA	66	72	NA	NA	NA
History of KS, %	NA	NA	49	67	27	25	25
HAART, %	NA	NA	69	78	NA	20 (1 of 5)	NA
Receiving chemotherapy, %	NA	61	88	79	73	75	100
CR rate, %	NA	NA	56	41	42	NA	NA
Median follow-up duration	4 mo	NA	10 y	3.8 y	NA	11 mo	1.1 mo
Median OS	NA	0.4 y	10.2 mo	6.2 mo	6 mo	11 mo	0.8 mo
1-y OS rate, %	30	NA	NA	39.3	NA	40	0
3-y OS rate, %	18	NA	NA	NA	NA	40	0
5-y OS rate, %	17	28	NA	NA	NA	40	0

Dashes indicate that patients were not included.

CR, complete response; HAART, highly active antiretroviral therapy; NA, not available.

chemotherapy that was the same as the current treatment regimens. In a previous retrospective analysis, receiving ART was actually identified as an independent favorable prognostic factor for OS.[55] Some patients who achieved complete remission after ART have been reported.[62-64] In addition to spontaneous regressions induced by discontinuation of MTX in patients with MTX-related lymphoproliferative disorder,[65] recovery of the host immune defense can improve the disease, at least in some patients with PEL. Additionally, PEL patients successfully treated with cidofovir, ganciclovir, or valganciclovir who could achieve CR have been reported.[63,66-69] Considering the possible beneficial effect of azidothymidine plus IFN-α treatment, which is effective in HTLV-1 infection, ART should be further investigated.[70]

To investigate whether ART against HIV infection improves treatment outcomes of PEL, Olszewski et al reported an analysis using the National Cancer Database (NCDB).[44] In 321 850 patients with NHL in the NCDB, 10 769 patients (3.4%) were HIV-seropositive. PEL developed in 106 of 10 769 HIV-infected patients (1%), whereas PEL occurred in only 75 of 311 081 HIV^- NHL patients (<1%). Clinical outcomes of HIV^+ patients with PEL receiving chemotherapy were consistent between 2004 and 2012; median OS was 0.7 years (95% confidence interval, 0.4-1.1 years) and 5-year OS was 28% (95% confidence interval, 17%-41%). El-Fattah also reported the clinical outcomes of 105 patients with PEL diagnosed in 18 US national cancer institutes based on the Surveillance, Epidemiology, and End Results (SEER) database between 2001 and 2012.[71] Eighty-two patients died of their disease, with median OS of 4.8 months. Forty patients (58.5%) died of HIV-associated complications, 21 patients (25.6%) died of progression of NHL, and 13 patients died of other causes. OS rates at 1, 3, and 5 years were 30%, 18%, and 17%, respectively. These findings indicate that avoiding deaths from HIV-associated complications resulting from immune recovery after the commencement of ART might be required to improve clinical outcomes of PEL. Although concurrent ART is advised in HIV-infected patients,[72] potential drug interactions between antiretroviral drugs and anticancer drugs should be considered.[73] Various anticancer drugs including cyclophosphamide and vincristine commonly used in lymphoma regimens are substrates of cytochrome P-450; thus, HIV medications such as ritonavir and cobicistat with strong effects on the cytochrome P-450 enzyme CYP3A4 should be avoided in patients who are undergoing lymphoma treatment.[74] Integrase strand-transfer inhibitor-based regimens that do not contain cobicistat are preferred in such cases. In patients with lymphoma who need to start ART, it is also reasonable to modify the timing of the start of ART after administration of the first cycle of chemotherapy.[73] Recently, ART given with dose-adjusted etoposide, prednisolone, vincristine, cyclophosphamide, and doxorubicin (DA-EPOCH) with rituximab (DA-EPOCH-R) or delayed until completion of

Table 2. Recent preclinical studies targeting activating pathway in PEL

Study ref.	Drug	Activity	Model	Key findings
77	Berberine	NF-κB inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	Induction of apoptosis and suppressed NF-κB activity by inhibiting IKK phosphorylation
83	Thioridazine	MALT1 inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	MALT1 inhibition induced a switch to lytic phase and the reduced growth and survival
78	3-AP	Ribonucleotide reductase inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	Induction of cell cycle arrest through the inhibition of NF-κB pathway
88	PEP005	Agonist of protein kinase C and activation of NF-κB	In vitro: PEL cell lines	Combination of PEP005 with BET inhibitor induced HHV8/KSHV lytic replication and the inhibition of IL6 production
91	PF-04691502, AKTi 1/2	PI3K/AKT/mTOR inhibition	In vitro: PEL cell lines	Combination of PI3K/AKT/mTOR inhibitor with a glycolytic inhibitor strengthened the cytotoxicity in PEL cells
89	PF-2341066	c-MET inhibition	In vitro: PEL cell lines	Induction of G2/M cell cycle arrest and apoptosis of PEL cells in vitro and in vivo
79	Everolimus	mTOR inhibition	In vitro: PEL cell lines	Induction of caspase-dependent apoptosis and downregulation of HHV8/KSHV latent antigen expression
93	Tenovin-6	SIRT1 and SIRT2 inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	Inhibition of cell proliferation and induction of apoptosis through AMPK inactivation
80	ABC294640	Sphingosine kinase 2 inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	Induction of apoptosis through the suppression of HHV8/KSHV-associated signal transduction
81	MLN4924	Neddylation inhibitor	In vitro: PEL cell lines	Induction of viral lytic protein expression
94	YM155	Survivin inhibition	In vitro: PEL cell line; PDX cell In vivo: PDX mouse model	Induction of apoptosis through the decreased MCL1 expression
90	PX-478	HIF-1α inhibition	In vitro: PEL cell lines	Induction of a dose-dependent decrease in HIF-1α mRNA and reduction of cell proliferation
92	Metformin	Reduction of intracellular ROS	In vitro: PEL cell lines	Induction of apoptosis through the downregulation of v-FLIP, the reduction of intracellular ROS, the activation of AMPK, the inhibition of mTOR, and the dephosphorylation of STAT3
82	Chloroquine	ER stress activation	In vitro: PEL cell lines In vivo: xenograft mouse model	Induction of ER stress-mediated apoptosis
87	IMiDs	Immunomodulation	In vitro: PEL cell lines	Preventing HHV8/KSHV-induced downregulation of MHC-1 surface expression

3-AP, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; AMPK, adenosine monophosphate–activated protein kinase; BET, bromodomain and extraterminal; ER, endoplasmic reticulum; IKZF, Ikaros family zinc finger protein; IMiD, immunomodulatory drug; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MCL1, myeloid cell leukemia 1; PDX, patient-derived xenograft; ROS, reactive oxygen species.

Table 2. (continued)

Study ref.	Drug	Activity	Model	Key findings
86	IMiDs	IKZF-IRF4-MYC axis inhibition	In vitro: PEL cell lines In vivo: xenograft mouse model	Induction of cell cycle arrest resulting in the suppression of IRF4 and the rapid degradation of IKZF1, but not IKZF3

3-AP, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; AMPK, adenosine monophosphate–activated protein kinase; BET, bromodomain and extraterminal; ER, endoplasmic reticulum; IKZF, Ikaros family zinc finger protein; IMiD, immunomodulatory drug; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MCL1, myeloid cell leukemia 1; PDX, patient-derived xenograft; ROS, reactive oxygen species.

chemotherapy was prospectively evaluated. Although concurrent ART was not associated with improved survival, concurrent ART was well tolerated and allowed for faster immune recovery.[75] According to the current National Comprehensive Cancer Network (NCCN) guideline, the use of chemotherapy regimens such as DA-EPOCH, cyclophosphamide, doxorubicin, and etoposide, and CHOP combined with ART is recommended for AIDS-related B-cell lymphoma. However, as discussed in "Clinical outcomes of patient series," the outcomes of such chemotherapies are still unsatisfactory, and an effort to further improve outcomes is needed.

Prognostic factors

The data on prognostic factors in patients with PEL are limited due to reports from small case series. Two previous retrospective studies that evaluated prognostic factors in PEL have been reported. The analysis of 28 patients reported by Boulanger et al showed poor performance status and absence of ART before PEL diagnosis as adverse prognostic factors.[55] A retrospective analysis of 104 patients with HHV8/KSHV$^+$ PEL demonstrated that patients with only 1 body cavity involved had a median OS of 18 months vs 4 months in patients with >1 cavity involved ($P = .003$). When evaluating patients with only 1 cavity involved, patients with pericardial involvement had a longer median OS than patients with pleural or peritoneal involvement, possibly due to the size and volume of the body cavity reflecting the tumor burden (40, 27, and 5 months, respectively; $P = .04$).[76]

Emerging therapies

With the poor clinical outcomes of existing strategies, the development of targeted therapy for activating pathways in PEL such as NF-κB, JAK/STAT, and phosphatidylinositol 3-kinase (PI3K)/AKT is continuously ongoing[77-83] (Table 2). Based on the finding of activation of the NF-κB pathway, the use of the proteasome inhibitor bortezomib in PEL has been investigated. Although inhibition of proliferation and induction of apoptosis of tumor cells could be confirmed in in vitro analyses, it was difficult to demonstrate efficacy of the drug in the clinical setting.[84] On the basis of the normal counterpart of PEL being post–germinal center B cells with plasmablastic differentiation, treatments using liposome-modified B-lymphocyte–induced maturation protein 1 small interfering RNA and immunomodulatory drugs (IMiDs) such as thalidomide, lenalidomide, and pomalidomide are being developed.[47,85] IMiDs are recognized as promising drugs in terms of their antiproliferative effects against the majority of PEL cell lines within clinically achievable concentrations by cell cycle arrest without any induction of lytic cycle reactivation. IMiDs suppressed IRF4 in a cereblon-dependent manner, resulting in rapid degradation of Ikaros (IKZF1), but not Aiolos (IKZF3), in PEL cell lines.[86] IMiDs also modified HHV8/KSHV-dependent downregulation of major histocompatibility complex type I (MHC-I) expression during lytic infection and expressions of ICAM-1 and B7-2 during latent infection, which allows recovery of T-cell and NK-cell immunity largely mediated by 2 HHV8/KSHV-encoded proteins, K3 and K5.[87] Actually, a clinical trial investigating the efficacy of modified DA-EPOCH-R combined with lenalidomide (EPOCH-R2) for patients with PEL is now being conducted at the National Cancer Institute (NCT02911142); it is the only PEL-specific study that translates the preclinical work to the clinical setting. In addition, the clinical efficacy of targeted therapy for the bromodomain and extraterminal (BET) family has been suggested; ingenol-3-angelate (PEP005), a US Food and Drug Administration (FDA)–approved drug for topical treatment of actinic keratosis, which is an agonist of protein kinase C and activated NF-κB, combined with JQ1, a BET inhibitor, synergistically induced lytic replication, resulting in an antitumor effect mediated by the inhibition of IL6 production.[88] The combination of IMiDs with a specific inhibitor against BRD4, a widely expressed transcriptional coactivator belonging to the BET family proteins, demonstrated a synergistic antiproliferative effect against PEL cell lines.[86] The hepatocyte growth factor (HGF)/c-MET pathway highly activated by HHV8/KSHV was shown to be a potential therapeutic target in PEL cells mediated by the downregulation of ribonucleoside-diphosphate reductase subunit M2 (RRM2).[89] Other therapies targeting the metabolism of tumor cells, such as the function of the Warburg effect, are also being developed. HHV8/KSHV infection promotes hypoxia-inducible factor-1α (HIF-1α) activity, which mediates much of the cellular response to hypoxia. HIF-1α knockdown in PEL cells leads to the reduction of both aerobic and anaerobic glycolysis, inhibiting the proliferation of PEL cells.[90] Another report also showed that a PI3K/mammalian target of rapamycin (mTOR) inhibitor and AKT inhibitor inhibited glycolysis in tumor cells, leading to a therapeutic effect on PEL cells.[91] Metformin, the most used drug for the treatment of type 2 diabetes mellitus, induced apoptosis in PEL cells that was correlated with intracellular reactive oxygen species, activation of adenosine monophosphate–activated protein kinase (AMPK), the inhibition of prosurvival pathways such as mTOR and STAT3, and the downregulation of v-FLIP.[92] Sirtuins (SIRT1), an NAD$^+$-dependent class III histone deacetylase regarded as metabolic sensors, are also required to sustain the proliferation and survival of PEL cells through AMPK activation, and tenovin-6, a SIRT1 and SIRT2 inhibitor, showed antitumor activity in PEL cells.[93] The majority of these preclinical studies investigated drug sensitivities and in vivo effects using established PEL cell lines and xenograft mouse models. Recently, Kojima et al reported the potential efficacy of a survivin inhibitor against PEL cells in vitro and in vivo obtained from a primary patient tumor cell–derived xenograft (PDX) mouse model. In their report, drug

candidates were picked out by a screening method from an existing drug library.[94] Using tumor cells from the PDX model could lead to the discovery of novel drugs with different modes of action from those identified using cell lines. The PDX model could be a powerful tool for developing new drug treatments.

Future perspectives

Most cases of PEL develop in HIV-infected individuals, and latent infection of HHV8/KSHV on the background of immune deficiency is involved in the disease mechanism. Recent progress regarding the efficacy of anti-PD1 antibodies in Hodgkin lymphoma and other types of NHL shows that the host immune response is doubtlessly involved in lymphomagenesis and the clinical efficacy of lymphoma treatment.[95-97] Moreover, monoclonal antibodies targeting PDL1, CD30, and CD38 are now used in clinical settings.[98] In particular, anti-CD38 monoclonal antibody daratumumab is highly effective in multiple myeloma.[99,100] Although daratumumab therapy for FL and DLBCL fell short of expectations, it might be promising for CD38 strongly positive PEL. Therefore, an attempt to improve the clinical outcomes of PEL developing on the basis of immunodeficiency should be quite a major challenge. With the recovery of the host immune defense by ART, the use of immunotherapy and/or the development of combination targeted therapy for effective inhibition of tumor proliferation and survival signaling will be vital to improve the clinical outcomes in PEL in the future.

Acknowledgments

The authors thank Koichi Oshima (Kurume University) and Shigeo Nakamura (Nagoya University) for preparing the pathological slides of PEL.

Authorship

Contribution: K.S., F.H., and H.K. wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profile: K.S., 0000-0002-1075-9219.

Correspondence: Kazuyuki Shimada, Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 4668550, Japan; e-mail: kshimada@med.nagoya-u.ac.jp.

Footnote

Submitted 9 March 2018; accepted 24 August 2018. Prepublished online as *Blood* First Edition paper, 28 August 2018; DOI 10.1182/blood-2018-03-791426.

REFERENCES

1. Said J, Cesarman E. Primary effusion lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2017.
2. Knowles DM, Inghirami G, Ubriaco A, Dalla-Favera R. 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(3):792-799.
3. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med*. 1995;332(18):1186-1191.
4. 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(2):645-656.
5. Pan ZG, Zhang QY, Lu ZB, et al. Extracavitary KSHV-associated large B-Cell lymphoma: a distinct entity or a subtype of primary effusion lymphoma? Study of 9 cases and review of an additional 43 cases [published correction appears in *Am J Surg Pathol*. 2013;37(3):458]. *Am J Surg Pathol*. 2012;36(8):1129-1140.
6. Carbone A, Gloghini A, Vaccher E, et al. Kaposi's sarcoma-associated herpesvirus/human herpesvirus type 8-positive solid lymphomas: a tissue-based variant of primary effusion lymphoma. *J Mol Diagn*. 2005;7(1):17-27.
7. Chadburn A, Hyjek E, Mathew S, Cesarman E, Said J, Knowles DM. KSHV-positive solid lymphomas represent an extra-cavitary variant of primary effusion lymphoma. *Am J Surg Pathol*. 2004;28(11):1401-1416.
8. Kim Y, Park CJ, Roh J, Huh J. Current concepts in primary effusion lymphoma and other effusion-based lymphomas. *Korean J Pathol*. 2014;48(2):81-90.
9. Brimo F, Michel RPP, Khetani K, Auger M. Primary effusion lymphoma: a series of 4 cases and review of the literature with emphasis on cytomorphologic and immunocytochemical differential diagnosis. *Cancer*. 2007;111(4):224-233.
10. Said JW, Shintaku IP, Asou H, et al. Herpesvirus 8 inclusions in primary effusion lymphoma: report of a unique case with T-cell phenotype. *Arch Pathol Lab Med*. 1999;123(3):257-260.
11. Kaplan LD. Human herpesvirus-8: Kaposi sarcoma, multicentric Castleman disease, and primary effusion lymphoma. *Hematology Am Soc Hematol Educ Program*. 2013;2013:103-108.
12. Castillo JJ, Bibas M, Miranda RN. The biology and treatment of plasmablastic lymphoma. *Blood*. 2015;125(15):2323-2330.
13. Harmon CM, Smith LB. Plasmablastic lymphoma: a review of clinicopathologic features and differential diagnosis. *Arch Pathol Lab Med*. 2016;140(10):1074-1078.
14. Schulz TF. The pleiotropic effects of Kaposi's sarcoma herpesvirus. *J Pathol*. 2006;208(2):187-198.
15. Mesri EA, Cesarman E, Boshoff C. Kaposi's sarcoma and its associated herpesvirus. *Nat Rev Cancer*. 2010;10(10):707-719.
16. Russo JJ, Bohenzky RA, Chien M-C, et al. Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proc Natl Acad Sci USA*. 1996;93(25):14862-14867.
17. Okada S, Goto H, Yotsumoto M. Current status of treatment for primary effusion lymphoma. *Intractable Rare Dis Res*. 2014;3(3):65-74.
18. Ballestas ME, Chatis PA, Kaye KM. Efficient persistence of extrachromosomal KSHV DNA mediated by latency-associated nuclear antigen. *Science*. 1999;284(5414):641-644.
19. Friborg J Jr, Kong W, Hottiger MO, Nabel GJ. p53 inhibition by the LANA protein of KSHV protects against cell death. *Nature*. 1999;402(6764):889-894.
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(10):1121-1127.
21. Thome M, Schneider P, Hofmann K, et al. Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature*. 1997;386(6624):517-521.
22. 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(25):9399-9404.
23. McCormick C, Ganem D. The kaposin B protein of KSHV activates the p38/MK2 pathway and stabilizes cytokine mRNAs. *Science*. 2005;307(5710):739-741.
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(4):833-843.

25. Muñoz-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(3):1518-1525.

26. Horenstein MG, Nador RG, Chadburn A, et al. Epstein-Barr virus latent gene expression in primary effusion lymphomas containing Kaposi's sarcoma-associated herpesvirus/human herpesvirus-8. *Blood*. 1997;90(3):1186-1191.

27. Fassone L, Bhatia K, Gutierrez M, et al. Molecular profile of Epstein-Barr virus infection in HHV-8-positive primary effusion lymphoma. *Leukemia*. 2000;14(2):271-277.

28. Szekely L, Chen F, Teramoto N, et al. Restricted expression of Epstein-Barr virus (EBV)-encoded, growth transformation-associated antigens in an EBV- and human herpesvirus type 8-carrying body cavity lymphoma line. *J Gen Virol*. 1998;79(Pt 6):1445-1452.

29. Liu WR, Shipp MA. Signaling pathways and immune evasion mechanisms in classical Hodgkin lymphoma. *Hematology Am Soc Hematol Educ Program*. 2017;2017:310-316.

30. Goodman A, Patel SP, Kurzrock R. PD-1-PD-L1 immune-checkpoint blockade in B-cell lymphomas. *Nat Rev Clin Oncol*. 2017;14(4):203-220.

31. Chen BJ, Chapuy B, Ouyang J, et al. PD-L1 expression is characteristic of a subset of aggressive B-cell lymphomas and virus-associated malignancies. *Clin Cancer Res*. 2013;19(13):3462-3473.

32. Host KM, Jacobs SR, West JA, et al. Kaposi's sarcoma-associated herpesvirus increases PD-L1 and proinflammatory cytokine expression in human monocytes. *MBio*. 2017;8(5):e00917.

33. Wies E, Mori Y, Hahn A, et al. The viral interferon-regulatory factor-3 is required for the survival of KSHV-infected primary effusion lymphoma cells. *Blood*. 2008;111(1):320-327.

34. Fan W, Bubman D, Chadburn A, Harrington WJ Jr, Cesarman E, Knowles DM. Distinct subsets of primary effusion lymphoma can be identified based on their cellular gene expression profile and viral association. *J Virol*. 2005;79(2):1244-1251.

35. Jenner RG, Maillard K, Cattini N, et al. Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphoma has a plasma cell gene expression profile. *Proc Natl Acad Sci USA*. 2003;100(18):10399-10404.

36. 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(10):4115-4121.

37. Punj V, Matta H, Chaudhary PM. A computational profiling of changes in gene expression and transcription factors induced by vFLIP K13 in primary effusion lymphoma. *PLoS One*. 2012;7(5):e37498.

38. O'Hara AJ, Wang L, Dezube BJ, Harrington WJ Jr, Damania B, Dittmer DP. Tumor suppressor microRNAs are underrepresented in primary effusion lymphoma and Kaposi sarcoma. *Blood*. 2009;113(23):5938-5941.

39. Yanagisawa Y, Sato Y, Asahi-Ozaki Y, et al. Effusion and solid lymphomas have distinctive gene and protein expression profiles in an animal model of primary effusion lymphoma. *J Pathol*. 2006;209(4):464-473.

40. Gloghini A, Volpi CC, Caccia D, et al. Primary effusion lymphoma: secretome analysis reveals novel candidate biomarkers with potential pathogenetic significance. *Am J Pathol*. 2014;184(3):618-630.

41. Yang D, Chen W, Xiong J, Sherrod CJ, Henry DH, Dittmer DP. Interleukin 1 receptor-associated kinase 1 (IRAK1) mutation is a common, essential driver for Kaposi sarcoma herpesvirus lymphoma [published correction appears in *Proc Natl Acad Sci USA*. 2015;112(18):E2412]. *Proc Natl Acad Sci USA*. 2014;111(44):E4762-E4768.

42. Wagener R, Alexandrov LB, Montesinos-Rongen M, et al. Analysis of mutational signatures in exomes from B-cell lymphoma cell lines suggest APOBEC3 family members to be involved in the pathogenesis of primary effusion lymphoma. *Leukemia*. 2015;29(7):1612-1615.

43. 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(21):3948-3954.

44. Olszewski AJ, Fallah J, Castillo JJ. Human immunodeficiency virus-associated lymphomas in the antiretroviral therapy era: analysis of the National Cancer Data Base. *Cancer*. 2016;122(17):2689-2697.

45. Dotti G, Fiocchi R, Motta T, et al. Primary effusion lymphoma after heart transplantation: a new entity associated with human herpesvirus-8. *Leukemia*. 1999;13(5):664-670.

46. 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(7):444-449.

47. Antar A, El Hajj H, Jabbour M, et al. Primary effusion lymphoma in an elderly patient effectively treated by lenalidomide: case report and review of literature. *Blood Cancer J*. 2014;4:e190.

48. Teruya-Feldstein J, Zauber P, Setsuda JE, et al. Expression of human herpesvirus-8 oncogene and cytokine homologues in an HIV-seronegative patient with multicentric Castleman's disease and primary effusion lymphoma. *Lab Invest*. 1998;78(12):1637-1642.

49. Klepfish A, Sarid R, Shtalrid M, Shvidel L, Berrebi A, Schattner A. Primary effusion lymphoma (PEL) in HIV-negative patients--a distinct clinical entity. *Leuk Lymphoma*. 2001;41(3-4):439-443.

50. Ichinohasama R, Miura I, Kobayashi N, et al. Herpes virus type 8-negative primary effusion lymphoma associated with PAX-5 gene rearrangement and hepatitis C virus: a case report and review of the literature. *Am J Surg Pathol*. 1998;22(12):1528-1537.

51. Alexanian S, Said J, Lones M, Pullarkat ST. KSHV/HHV8-negative effusion-based lymphoma, a distinct entity associated with fluid overload states. *Am J Surg Pathol*. 2013;37(2):241-249.

52. Wu W, Youm W, Rezk SA, Zhao X. Human herpesvirus 8-unrelated primary effusion lymphoma-like lymphoma: report of a rare case and review of 54 cases in the literature. *Am J Clin Pathol*. 2013;140(2):258-273.

53. Nakatsuka S, Yao M, Hoshida Y, Yamamoto S, Iuchi K, Aozasa K. Pyothorax-associated lymphoma: a review of 106 cases. *J Clin Oncol*. 2002;20(20):4255-4260.

54. Lam GK, Abdelhaleem M, Somers GR, Roifman C, Read S, Abla O. Primary effusion lymphoma (PEL)-like lymphoma in a child with congenital immunodeficiency. *Pediatr Blood Cancer*. 2016;63(9):1674-1676.

55. Boulanger E, Gérard 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(19):4372-4380.

56. Boulanger E, Agbalika F, Maarek O, et al. A clinical, molecular and cytogenetic study of 12 cases of human herpesvirus 8 associated primary effusion lymphoma in HIV-infected patients. *Hematol J*. 2001;2(3):172-179.

57. Guillet S, Gérard L, Meignin V, et al. Classic and extracavitary primary effusion lymphoma in 51 HIV-infected patients from a single institution. *Am J Hematol*. 2016;91(2):233-237.

58. Evans WE, Pratt CB. Effect of pleural effusion on high-dose methotrexate kinetics. *Clin Pharmacol Ther*. 1978;23(1):68-72.

59. 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(4):328-330.

60. 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(2):67-73.

61. Bryant A, Milliken S. Successful reduced-intensity conditioning allogeneic HSCT for HIV-related primary effusion lymphoma. *Biol Blood Marrow Transplant*. 2008;14(5):601-602.

62. Oksenhendler E, Clauvel JP, Jouveshomme S, Davi F, Mansour G. Complete remission of a primary effusion lymphoma with antiretroviral therapy. *Am J Hematol*. 1998;57(3):266.

63. Hocqueloux L, Agbalika F, Oksenhendler E, Molina JM. Long-term remission of an AIDS-related primary effusion lymphoma with antiviral therapy. *AIDS*. 2001;15(2):280-282.

64. Ripamonti D, Marini B, Rambaldi A, Suter F. Treatment of primary effusion lymphoma with highly active antiviral therapy in the setting of HIV infection. *AIDS*. 2008;22(10):1236-1237.

65. Salloum E, Cooper DL, Howe G, et al. Spontaneous regression of lymphoproliferative disorders in patients treated with

methotrexate for rheumatoid arthritis and other rheumatic diseases. *J Clin Oncol*. 1996; 14(6):1943-1949.

66. Pereira R, Carvalho J, Patrício C, et al. Sustained complete remission of primary effusion lymphoma with adjunctive ganciclovir treatment in an HIV-positive patient. *BMJ Case Rep*. 2014;2014.

67. Moyo TK, Richards KL, Damania B. Use of cidofovir for the treatment of HIV-negative human herpes virus-8-associated primary effusion lymphoma. *Clin Adv Hematol Oncol*. 2010;8(5):372-374.

68. Luppi M, Trovato R, Barozzi P, et al. Treatment of herpesvirus associated primary effusion lymphoma with intracavity cidofovir. *Leukemia*. 2005;19(3):473-476.

69. Marquet J, Velazquez-Kennedy K, López S, Benito A, Blanchard MJ, Garcia-Vela JA. Case report of a primary effusion lymphoma successfully treated with oral valganciclovir after failing chemotherapy. *Hematol Oncol*. 2018;36(1):316-319.

70. Ghosh SK, Wood C, Boise LH, et al. Potentiation of TRAIL-induced apoptosis in primary effusion lymphoma through azidothymidine-mediated inhibition of NF-kappa B. *Blood*. 2003;101(6):2321-2327.

71. El-Fattah MA. Clinical characteristics and survival outcome of primary effusion lymphoma: a review of 105 patients. *Hematol Oncol*. 2017;35(4):878-883.

72. Barta SK, Xue X, Wang D, et al. Treatment factors affecting outcomes in HIV-associated non-Hodgkin lymphomas: a pooled analysis of 1546 patients. *Blood*. 2013;122(19): 3251-3262.

73. Yarchoan R, Uldrick TS. HIV-associated cancers and related diseases. *N Engl J Med*. 2018;378(11):1029-1041.

74. Rudek MA, Flexner C, Ambinder RF. Use of antineoplastic agents in patients with cancer who have HIV/AIDS. *Lancet Oncol*. 2011; 12(9):905-912.

75. Tan CRC, Barta SK, Lee J, Rudek MA, Sparano JA, Noy A. Combination antiretroviral therapy accelerates immune recovery in patients with HIV-related lymphoma treated with EPOCH: a comparison within one prospective trial AMC034. *Leuk Lymphoma*. 2018;59(8):1851-1860.

76. Castillo JJ, Shum H, Lahijani M, Winer ES, Butera JN. Prognosis in primary effusion lymphoma is associated with the number of body cavities involved. *Leuk Lymphoma*. 2012;53(12):2378-2382.

77. Goto H, Kariya R, Shimamoto M, et al. Antitumor effect of berberine against primary effusion lymphoma via inhibition of NF-κB pathway. *Cancer Sci*. 2012;103(4):775-781.

78. Dai L, Lin Z, Qiao J, Chen Y, Flemington EK, Qin Z. Ribonucleotide reductase represents a novel therapeutic target in primary effusion lymphoma. *Oncogene*. 2017;36(35): 5068-5074.

79. Mohanty S, Kumar A, Das P, Sahu SK, Choudhuri T. Multi-targeted therapy of everolimus in Kaposi's sarcoma associated herpes virus infected primary effusion lymphoma. *Apoptosis*. 2017;22(9):1098-1115.

80. Dai L, Bai A, Smith CD, Rodriguez PC, Yu F, Qin Z. ABC294640, a novel sphingosine kinase 2 inhibitor, induces oncogenic virus-infected cell autophagic death and represses tumor growth. *Mol Cancer Ther*. 2017; 16(12):2724-2734.

81. Chang PJ, Chen LW, Chen LY, Hung CH, Shih YJ, Wang SS. Effects of the NEDD8-activating enzyme inhibitor MLN4924 on lytic reactivation of Kaposi's sarcoma-associated herpesvirus. *J Virol*. 2017;91(19).

82. Masud Alam M, Kariya R, Kawaguchi A, Matsuda K, Kudo E, Okada S. Inhibition of autophagy by chloroquine induces apoptosis in primary effusion lymphoma in vitro and in vivo through induction of endoplasmic reticulum stress. *Apoptosis*. 2016;21(10): 1191-1201.

83. Bonsignore L, Passelli K, Pelzer C, et al. A role for MALT1 activity in Kaposi's sarcoma-associated herpes virus latency and growth of primary effusion lymphoma. *Leukemia*. 2017;31(3):614-624.

84. 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(29): 13069-13074.

85. Riva G, Lagreca I, Mattiolo A, et al. Antineoplastic effects of liposomal short interfering RNA treatment targeting BLIMP1/PRDM1 in primary effusion lymphoma. *Haematologica*. 2015;100(11):e467-e470.

86. Gopalakrishnan R, Matta H, Tolani B, Triche T Jr, Chaudhary PM. Immunomodulatory drugs target IKZF1-IRF4-MYC axis in primary effusion lymphoma in a cereblon-dependent manner and display synergistic cytotoxicity with BRD4 inhibitors. *Oncogene*. 2016; 35(14):1797-1810.

87. Davis DA, Mishra S, Anagho HA, et al. Restoration of immune surface molecules in Kaposi sarcoma-associated herpes virus infected cells by lenalidomide and pomalidomide. *Oncotarget*. 2017;8(31):50342-50358.

88. Zhou F, Shimoda M, Olney L, et al. Oncolytic reactivation of KSHV as a therapeutic approach for primary effusion lymphoma. *Mol Cancer Ther*. 2017;16(11):2627-2638.

89. Dai L, Trillo-Tinoco J, Cao Y, et al. Targeting HGF/c-MET induces cell cycle arrest, DNA damage, and apoptosis for primary effusion lymphoma. *Blood*. 2015;126(26):2821-2831.

90. Shrestha P, Davis DA, Veeranna RP, Carey RF, Viollet C, Yarchoan R. Hypoxia-inducible factor-1 alpha as a therapeutic target for primary effusion lymphoma. *PLoS Pathog*. 2017;13(9):e1006628.

91. Mediani L, Gibellini F, Bertacchini J, et al. Reversal of the glycolytic phenotype of primary effusion lymphoma cells by combined targeting of cellular metabolism and PI3K/Akt/ mTOR signaling. *Oncotarget*. 2016;7(5): 5521-5537.

92. Granato M, Gilardini Montani MS, Romeo MA, et al. Metformin triggers apoptosis in PEL cells and alters bortezomib-induced unfolded protein response increasing its cytotoxicity and inhibiting KSHV lytic cycle activation. *Cell Signal*. 2017;40:239-247.

93. He M, Tan B, Vasan K, et al. SIRT1 and AMPK pathways are essential for the proliferation and survival of primary effusion lymphoma cells. *J Pathol*. 2017;242(3):309-321.

94. Kojima Y, Hayakawa F, Morishita T, et al. YM155 induces apoptosis through proteasome-dependent degradation of MCL-1 in primary effusion lymphoma. *Pharmacol Res*. 2017;120:242-251.

95. Kwong YL, Chan TSY, Tan D, et al. PD1 blockade with pembrolizumab is highly effective in relapsed or refractory NK/T-cell lymphoma failing l-asparaginase. *Blood*. 2017;129(17):2437-2442.

96. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372(4):311-319.

97. Chen R, Zinzani PL, Fanale MA, et al; KEYNOTE-087. Phase II study of the efficacy and safety of pembrolizumab for relapsed/refractory classic Hodgkin lymphoma. *J Clin Oncol*. 2017;35(19):2125-2132.

98. Bhatt S, Ashlock BM, Natkunam Y, et al. CD30 targeting with brentuximab vedotin: a novel therapeutic approach to primary effusion lymphoma. *Blood*. 2013;122(7):1233-1242.

99. Palumbo A, Chanan-Khan A, Weisel K, et al; CASTOR Investigators. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;375(8):754-766.

100. Dimopoulos MA, Oriol A, Nahi H, et al; POLLUX Investigators. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;375(14):1319-1331.