発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Inamoto Y., Suzuki R., Kuwatsuka Y., Yasuda T., Takahashi T., Tsujimura A., Sugimoto K., Oba T., Terakura S., Atsuta Y., Murata M., Ito M., Kodera Y. and Miyamura K.	Long-term outcome after bone marrow transplantation for aplastic anemia using cyclophosphamide and total lymphoid irradiation as conditioning regimen.	Biol Blood Marrow Transplant	14	43-49	2008
Nomura Y., Karube K., Suzuki R., Ying G., Takeshita M., Hirose S., Nakamura S., Yoshino T., Kikuchi M. and Ohshima K.	High-grade mature B-cell lymphoma with Burkitt-like morphology: results of a clinicopathologic study of 72 Japanese patients.	Cancer Sci	99	246-252	2008
Narimatsu H., Yokozawa T., Iida H., Tsuzuki M., Hayakawa M., Takeo T., Iino M., Ichihashi T., Kato C., Sawamoto A., Sao H., Yanada M., Emi N., Kiyoi H., Yamaguchi T., Naoe T., Suzuki R. and Sugiura I.	Clinical characteristics and outcomes in patients with t(8;21) acute myeloid leukemia in Japan.	Leukemia	22	428-432	2008
Yamaguchi M., Suzuki R., Kwong Y.-L., Kim W.S., Hasegawa Y., Izutsu K., Suzumiya J., Okamura T., Nakamura S., Kawa K. and Oshimi K.	Phase I study of SMILE chemotherapy for advanced-stage or relapsed/refractory extranodal NK/T-cell lymphoma/leukemia.	Cancer Sci	99	1016-1020	2008
Yamaguchi M., Nakamura N., Suzuki R., Kagami Y., Okamoto M., Ichinohasama R., Yoshino T., Suzumiya J., Murase T., Miura I., Ohshima K., Nishikori M., Tamaru J., Taniwaki M., Hirano M., Morishima Y., Ueda R., Shiku H. and Nakamura S.	De novo CD5+ diffuse large B-cell lymphoma: results of a detail clinicopathologic review in 120 patients.	Haematologica	93	1195-1202	2008
Suzuki R., Takeuchi K., Ohshima K. and Nakamura S.	Extranodal NK/T-cell lymphoma: diagnosis and treatment cues.	Hematol Oncol	26	66-72	2008
Kimura H., Ito Y., Suzuki R. and Nishiyama Y.	Measuring Epstein-Barr virus (EBV) load: its significance and application in each EBV-associated disease.	Rev Med Virol	18	305-319	2008
Narimatsu H., Iino M., Ichihashi T., Yokozawa T., Hayakawa M., Kiyoi H., Takeo T., Sawamoto A., Iida H., Tsuzuki M., Yanada M., Naoe T., Suzuki R. and Sugiura I.	Clinical significance of minimal residual disease in patients with t(8;21) acute myeloid leukemia in Japan.	Int J Hematol	88	154-158	2008
Ennishi D., Takeuchi K., Yokoyama M., Asai H., Mishima Y., Terui Y., Takahashi S., Komatsu H., Ikeda K., Yamaguchi M., Suzuki R., Tanimoto M. and Hatake K.	CD5 expression is potentially predictive of poor outcome among biomarkers in patients with diffuse large B-cell lymphoma receiving rituximab plus CHOP therapy.	Ann Oncol	19	1921-1926	2008
Lee J., Au W.Y., Park M.J., Suzumiya J., Nakamura S., Kameoka J.-I., Sakai C., Oshimi K., Kwong Y.-L., Liang R., Yiu H., Wong K.-H., Cheng H.-C., Ryoo B.-Y., Suh C., Ko Y.H., Kim K., Lee J.-W., Kim W.S. and Suzuki R.	Autologous hematopoietic stem cell transplantation in extranodal NK/T-cell lymphoma: a multinational, multicenter, matched controlled study.	Biol Blood Marrow Transplant	14	1356-1364	2008
Atsuta Y., Suzuki R., Nagamura-Inoue T., Taniguchi S., Takahashi S., Kai S., Sakamaki H., Kouzai Y., Kasai M., Fukuda T., Azuma H., Takanashi M., Okamoto S., Tsuchida M., Kawa K., Morishima Y., Kodera Y., and Kato S. for the Japan	Marrow Donor Program and the Japan Cord Blood Bank Network. Disease-specific analyses of unrelated cord blood transplant compared with unrelated bone marrow transplant in adult patients with acute leukemia.	Blood	113	1631-1638	2009

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Kuwatsuka Y., Miyamura K., Suzuki R., Kasai M., Maruta A., Ogawa H., Tanosaki R., Takahashi S., Koda K., Yago K., Atsuta Y., Yoshida T., Sakamaki H. and Kodera Y.	Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes.	Blood	113	2096-2103	2009
Suzuki R., Suzumiya J. and Oshimi K	Differences between nasal and extra-nasal NK/T-cell lymphoma.	Blood	113	6260-6261	2009
Inamoto Y., Ito M., Suzuki R., Nishida T., Nishiwaki S., Iida H., Kohno A., Murata M., Sawa M., Oba T., Yanada M., Naoe T., Ichihashi R., Fujino M., Yamaguchi T., Morishita Y., Hirabayashi N., Kodera Y. and Miyamura K.	Clinicopathological manifestations and treatment of intestinal transplant-associated microangiopathy (i-TAM).	Bone Marrow Transplant	44	43-49	2009
Lee S.Y., Kumano K., Nakazaki K., Sanada M., Matsumoto A., Yamamoto G., Nannya Y., Suzuki R. , Ota S., Ota Y., Izutsu K., Sakata-Yanagimoto M., Hangaishi A., Yagita H., Fukayama M., Seto M., Kurokawa M., Ogawa S. and Chiba S.	Gain-of-function mutations and copy number increases of Notch2 in diffuse large B-cell lymphoma.	Cancer Sci	100	920-926	2009
Shimada K., and Suzuki R.	Concurrent chemoradiotherapy for limited-stage extranodal NK/T-cell lymphoma, nasal type.	J Clin Oncol		[in press]	2010
Hyo R., Tomita N., Takeuchi K., Aoshima T., Fujita A., Kuwabara H., Hashimoto C., Takemura S., Taguchi J., Sakai R., Fujita H., Fujisawa S., Ogawa K., Motomura S., Suzuki R. and Ishigatsubo Y.	The therapeutic effect of rituximab on CD5-positive and CD5-negative diffuse large B-cell lymphoma.	Hematol Oncol		[in press]	2010
Inamoto Y., Murata M., Katsumi A., Kuwatsuka Y., Tsujimura A., Ishikawa Y., Sugimoto K., Onizuka M., Terakura S., Nishida T., Kanie T., Taji H., Iida H., Suzuki R., Abe A., Kiyoi H., Matsushita T., Miyamura K., Kodera Y., Naoe T.	Donor single nucleotide polymorphism in the CCR9 gene affects the incidence of skin GVHD.	Bone Marrow Transplant		[in press]	2010
Suzuki R., Suzumiya J., Yamaguchi M., Nakamura S., Kameoka J., Kojima H., Abe M., Kinoshita T., Yoshino T., Iwatsuki K., Kagami Y., Tsuzuki T., Kurokawa M., Ito K., Kawa K., and Oshimi K. for The NK-cell Tumor Study Group.	Prognostic factors for mature natural killer (NK)-cell neoplasms: aggressive NK-cell leukemia and extranodal NK-cell lymphoma, nasal-type.	Ann Oncol		[in press]	2010
Suzuki R., Ohtake S., Takeuchi J., Nagai M., Kodera Y., Hamaguchi M., Miyawaki S., Karasuno T., Shimodaira S., Ohno R., Nakamura S. and Naoe T.	The clinical characteristics of CD7+ CD56+ acute myeloid leukemias other than M0.	Int J Hematol		[in press]	2010
Yoshimi A., Suzuki R., Atsuta Y., Iida M., Lu D.-P., Tong W., Ghavamzadeh A., Alimoghaddam K., Lie A.K.W., Liang R., Chan L.L., Haipeng L., Tan P.-L., Hwang W.Y.K., Chiou T.-J., Chen P.-M., Binh T.V., Minh N.N., Min C.-K., Hwang T.-J., and Kodera Y. on behalf of Asia-Pacific Blood and Marrow Transplantation Group (APBMT)	Hematopoietic stem cell transplantation activity in Asia: A report from the Asia-Pacific Blood and Marrow Transplantation Group.	Bone Marrow Transplant		[in press]	2010

発表者氏名	論文タイトル名	発表誌名	巻名	ページ	出版年
Asano N., Suzuki R., Oshima K., Kagami Y., Ishida F., Yoshino T., Morishima Y. and Nakamura S.	Linkage of expression of chemokine receptors (CXCR3 and CCR4) and cytotoxic molecules in peripheral T-cell lymphoma, unspecified and ALK-negative anaplastic large cell lymphoma.	Int J Hematol		[in press]	2010

Ⅲ. 研究成果の刊行物・別刷

bjh review

Progress in understanding and managing natural killer-cell malignancies

Kazuo Oshimi

Department of Haematology, Juntendo University School of Medicine, Tokyo, Japan

Summary

The World Health Organization classification of haematolymphoid tumours recognizes three categories of natural killer (NK)-cell neoplasms: blastic NK-cell lymphoma, aggressive NK-cell leukaemia, and extranodal NK/T-cell lymphoma, nasal-type. Recent studies indicate that $CD4^+CD56^+$ blastic NK-cell lymphoma is of plasmacytoid dendritic cell origin, and true tumours of precursor NK-cell origin may be present mainly in the $CD4^-CD56^+$ subset. Myeloid/NK-cell precursor acute leukaemia may also develop from precursor NK cells. However, because the developmental pathway of normal NK cells is not well understood, tumours of precursor NK-cell origin are not clearly identified. Among mature NK-cell tumours, extranodal NK/T-cell lymphoma is relatively common in Asia and Latin America. In localized disease, chemoradiotherapy seems to be promising, and in advanced disease, new combination chemotherapies are under active investigation. Aggressive NK-cell leukaemia is rare and has a poor prognosis. Because NK-cell neoplasms are rare and difficult to manage, rigorous studies are required for their understanding and management.

Keywords: myeloid/NK-cell precursor acute leukaemia, precursor NK-lymphoblastic leukaemia/lymphoma, aggressive NK-cell leukaemia, extranodal NK/T-cell lymphoma, chronic NK-cell lymphocytosis.

Introduction

Natural killer (NK) cells are innate immune lymphocytes that mediate two major functions: recognition and lysis of tumour and virus-infected cells, and production of immunoregulatory cytokines. Mature NK cells are characterized by (i) large granular lymphocyte (LGL) morphology, (ii) $CD3^-CD56^+$ phenotype, (iii) cytotoxic function against major histocompatibility complex class I-deficient target cells without prior sensitization and (iv) germ-line configuration T-cell receptor (TCR) genes (Hercend & Schmidt, 1988; Trinchieri, 1989; Robertson & Ritz, 1990).

To delineate NK cell-lineage tumours that conceivably originate from precursor and mature NK cells, understanding of the developmental pathway of normal NK cells is crucial. Here, the developmental pathway of normal NK cells is described, followed by the clinical features and treatment of tumours that conceivably originate from precursor and mature NK cells, with special emphasis on their relationship with possible normal NK counterparts.

Developmental pathway of normal NK cells

Our understanding of human NK-cell development lags far behind that of B- and T-cell development. $CD34^+$ haematopoietic stem cells in the bone marrow differentiate into common myeloid progenitors and common lymphoid progenitors (CLP), and CLP differentiate into B cells, dendritic cells (DC), T cells and NK cells (Fig 1) (Spits *et al*, 1998; Freud & Caligiuri, 2006). DC derived from CLP are considered to be plasmacytoid DC (pDC), but not conventional DC (cDC) (Liu, 2001). However, the developmental pathway of DC is still a matter of debate, and the ontogeny of pDC and cDC is not clearly identified.

In NK-cell development, CLP subsequently differentiate into stage 1 progenitor NK (pro-NK) cells, stage 2 precursor NK (pre-NK) cells, stage 3 committed immature NK (iNK) cells, stage 4 mature NK (mNK) cells and finally stage 5 mNK cells (Fig 1) (Freud & Caligiuri, 2006). Stage 1 and stage 2 cells also differentiate into T cells and DC, and therefore are tripotential T/NK/DC common progenitors (Freud *et al*, 2006). These findings suggest a potential for substantial overlap in phenotypic and functional characteristics between NK cells, T cells and DC (Spits & Lanier, 2007). DC differentiated from stage 1 pro-NK and stage 2 pre-NK cells seem to be cDC, but not pDC (Marquez *et al*, 1998).

Stage 4 and stage 5 mNK cells are characterized by bright CD56 ($CD56^{bright}$) and dim CD56 ($CD56^{dim}$) expression respectively (Fig 2) (Nagler *et al*, 1989; Cooper *et al*, 2001). Stage 4 $CD56^{bright}$ mNK cells are $CD16^{dim/neg}$, produce abundant cytokines, such as interferon (IFN)-γ, tumour

Correspondence: Kazuo Oshimi, MD, Department of Haematology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. E-mail: oshimi@med.juntendo.ac.jp

First published online 3 October 2007
doi:10.1111/j.1365-2141.2007.06835.x

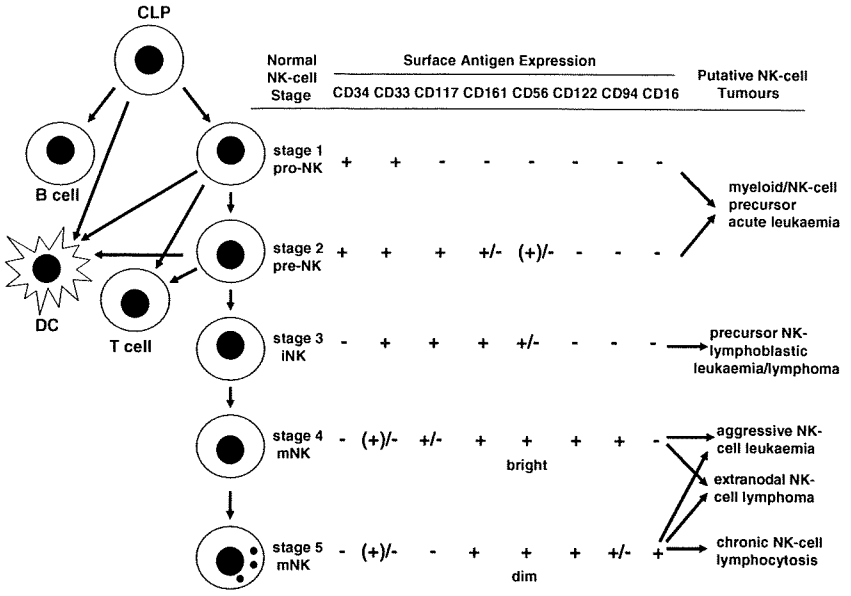

Fig 1. Developmental pathway of normal natural killer (NK) cells and putative NK-lineage tumours arising from precursor and mature NK cells. $CD34^+$ haematopoietic stem cells differentiate into common myeloid progenitors and common lymphoid progenitors (CLP). CLP then differentiate into B cells, dendritic cells (DC), T cells, and NK cells. In the NK-cell developmental pathway, CLP differentiate into stage 1 progenitor NK (pro-NK) cells, stage 2 precursor NK (pre-NK) cells, stage 3 committed immature NK (iNK) cells, stage 4 mature NK (mNK) cells, and finally stage 5 mNK cells (Freud & Caligiuri, 2006). Stage 1 and stage 2 cells also differentiate into T cells and DC, and therefore are tripotential T/NK/DC common progenitors. Myeloid/NK-cell precursor acute leukaemia arises conceivably from stage 1 pro-NK and stage 2 pre-NK cells, and 'precursor NK-lymphoblastic leukaemia/lymphoma' (the name proposed here in this review) conceivably arises from stage 3 iNK cells, or a stage between stage 3 iNK and stage 4 mNK cells. Aggressive NK-cell leukaemia, nasal-type NK-cell lymphoma, and chronic NK-cell lymphocytosis develop from stage 4 mNK and/or stage 5 mNK cells.

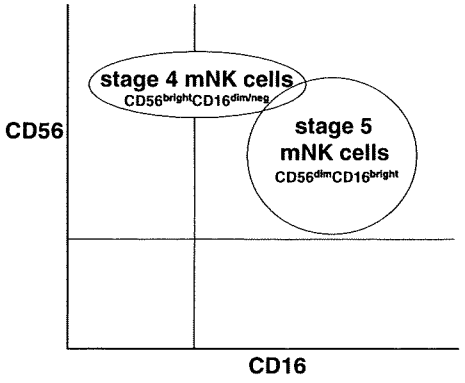

Fig 2. Co-expression of CD56 and CD16 on mature natural killer (NK) cells. NK cells isolated from normal donor peripheral blood were stained with anti-CD56 and anti-CD16 monoclonal antibodies (Nagler *et al*, 1989). Stage 4 mature NK (mNK) cells are characterized by $CD56^{bright}CD16^{dim/neg}$ expression, production of abundant cytokines, and low natural and antibody-dependent cytotoxicity. They are present mainly in the lymph nodes and tonsils. Stage 5 mNK cells are characterized by $CD56^{dim}CD16^{bright}$ expression, production of low levels of cytokines, and potent natural and antibody-dependent cytotoxicity. They are present mainly in the peripheral blood and spleen (Nagler *et al*, 1989; Cooper *et al*, 2001; Fehniger *et al*, 2003; Ferlazzo *et al*, 2004).

necrosis factor (TNF)-α, and granulocyte–macrophage colony-stimulating factor, and are less effective mediators of natural cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC). By contrast, stage 5 $CD56^{dim}$ mNK cells are $CD16^{bright}$, produce low levels of cytokines, and are potent mediators of natural cytotoxicity and ADCC. Stage 4 $CD56^{bright}$ mNK cells are present mainly in the lymph nodes and tonsils, and stage 5 $CD56^{dim}$ mNK cells are present mainly in the peripheral blood and spleen (Fehniger *et al*, 2003; Ferlazzo *et al*, 2004). Upon IL-2 stimulation *in vitro*, stage 4 mNK cells display phenotypic and functional similarities to stage 5 mNK cells, implying that stage 4 mNK cells mature to stage 5 mNK cells *in vivo*. Thus, intermediate NK precursor cells traffic to secondary lymphoid tissues, and the terminal differentiation may take place in these tissues. Taking into account the organ distribution of lymphocytes, stage 4 mNK cells probably outnumber stage 5 mNK cells in the human body (Ferlazzo *et al*, 2004).

Tumours conceivably originating from NK-lineage cells

Based on progress in understanding normal NK cells, tumours putatively derived from NK-lineage cells have been proposed and characterized. To understand NK-cell and related tumours, see the following reviews: Oshimi (1996), Jaffe (1996), Kwong *et al* (1997), Chan (1997), Siu *et al* (2002), Oshimi (2003), Cheung *et al* (2003), Nava and Jaffe (2005), Suzuki (2005) and Kwong (2005).

The new World Health Organization (WHO) classification of haematolymphoid tumours recognizes three categories of NK cell neoplasms: blastic NK-cell lymphoma, aggressive NK-cell leukaemia, and extranodal NK/T-cell lymphoma, nasal type. Before the development of the WHO classification, we proposed six types of NK-lineage tumours in our NK-cell Tumour Study Group (Oshimi, 2000, 2003; Oshimi *et al*, 2005) to try to focus on and characterize pure NK-lineage tumours. In our proposal, tumours that conceivably originate from precursor NK cells included myeloid/NK-cell precursor acute leukaemia, precursor NK-cell acute lymphoblastic leukaemia (ALL) and blastic NK-cell lymphoma. Tumours of mature NK-cell origin included aggressive NK-cell leukaemia/lymphoma, nasal-type NK-cell lymphoma, and chronic NK-cell lymphocytosis, but the last disorder seems to be reactive in most cases. The characteristics of these tumours, based on our nationwide survey, were described by Oshimi *et al* (2005), and Fig 3 shows the overall survival curves of patients with these tumours.

Because the developmental pathway of normal NK cells and the characteristics of precursor NK cells are not fully understood, and the incidence of precursor NK-cell tumours is rare, the definition and characterization of these tumours are only provisional. Indeed, our subsequent analysis revealed few clinicopathological differences between precursor NK-cell ALL and blastic NK-cell lymphoma (Suzuki *et al*, 2005), and accordingly I would like to propose here the name 'precursor NK-lymphoblastic leukaemia/lymphoma' as a collective classification, based on the WHO nomenclature of precursor T- and B-lymphoblastic leukaemia/lymphoma. Further, considering the recent finding that $CD4^+CD56^+$ blastic NK-cell lymphoma is of pDC origin (reviewed by Garnache-Ottou *et al*, 2007), the name precursor NK-lymphoblastic leukaemia/lymphoma should be applied mainly to the non-T, non-B $CD4^-CD56^+$ phenotype.

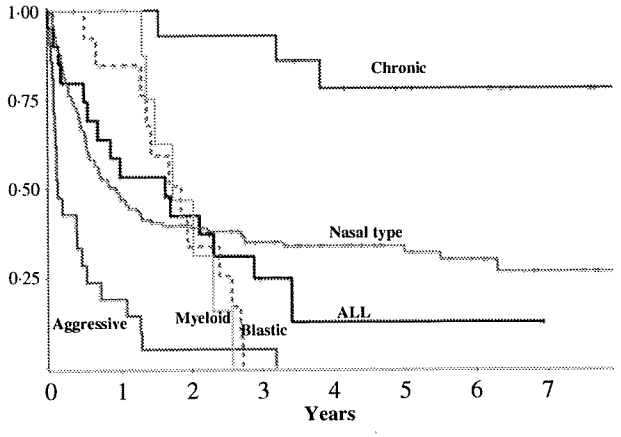

Fig 3. Overall survival curves of patients with natural killer (NK) cell-lineage tumours based on our nationwide survey. Before the development of the WHO classification, we proposed six types of NK-lineage tumours in our NK-cell Tumour Study Group, and tried to characterize their clinical features (Oshimi, 2000, 2003; Oshimi *et al*, 2005). In our proposal, tumours conceivably originating from precursor NK cells included myeloid/NK-cell precursor acute leukaemia (myeloid), precursor NK-cell acute lymphoblastic leukaemia (ALL), and blastic NK-cell lymphoma (blastic). Tumours of mature NK-cell origin included aggressive NK-cell leukaemia/lymphoma (aggressive), nasal-type NK-cell lymphoma (nasal type) and chronic NK-cell lymphocytosis (chronic).

Myeloid/NK-cell precursor acute leukaemia

One of the candidates for NK-cell tumours originating from precursor NK cells is myeloid/NK-cell precursor acute leukaemia. This disease was originally described by Suzuki *et al* (1997) and further characterized by Suzuki and Nakamura (1999). This is acute myeloid leukaemia (AML) M0 (French-American-British classification) co-expressing CD7 and CD56, and conceivably originated from $CD7^+CD33^+CD34^+$ stage 1 and stage 2 T/NK/DC tripotential progenitors. However, because CD7 and CD56 antigens are often expressed in AML cells, the possibility that this disease is of true myeloid cell origin cannot be ruled out. When purified tumour cells develop NK-, T- or DC-lineage features *in vitro* under different culture conditions, their common progenitor origin can be confirmed.

Diagnosis

Diagnosis is made when myeloperoxidase-negative and Sudan black B-negative blasts are present that are phenotypically $CD7^+CD56^+CD3^-CD34^+$ and myeloid antigens $(CD13 \text{ and/or } CD33)^+$. Their TCR-β (*TRB@*) and IgH (*IGH@*) genes should be in the germ-line configuration, but the TCR-δ gene (*TRD@*) may be rearranged (Spits *et al*, 1998). It is a rare disease and manifests as leukaemia or extramedullary lesions mainly involving lymph nodes and mediastinum with eventual leukaemic change. Because myeloid/NK-cell precursor acute leukaemia expresses myeloid antigens, it is difficult to differentiate it from other AML M0, i.e. AML M0 expressing either CD7 or CD56, or lacking both antigens. The characteristics of $CD7^+CD56^+$ AML M0, however, are markedly different from those of other AML M0 in terms of lower patient age, extramedullary disease presentation, different haematological manifestations, lack of 5q abnormalities, and poor prognosis (Suzuki *et al*, 2003). Therefore, the simultaneous expression of CD7 and CD56 antigens differentiates myeloid/NK-cell precursor acute leukaemia from other AML M0.

Scott *et al* (1994) described an $HLA\text{-}DR^+CD33^+CD56^+$ $CD16^-$ myeloid/NK cell acute leukaemia. Because this previously unrecognized form of acute leukaemia is positive for Sudan black B and myeloperoxidase, and various types of AML cells have the CD56 antigen, it is highly likely that this leukaemia is of myeloid origin. Induction of natural cytotoxicity after short-term *in vitro* culture of leukaemic cells cannot rule out an NK origin.

Pathogenesis

No recurrent karyotypic abnormalities have been reported, but some have 7p translocations or 3p abnormalities, suggesting that certain genes responsible for the pathogenesis of the disease might exist in these chromosomal regions (Suzuki & Nakamura, 1999). Epstein-Barr virus (EBV) is not detected in the tumour cells.

Treatment and prognosis

Therapeutic response to AML chemotherapy regimens seems to be better than that to ALL regimens. Relapse is frequent, and the prognosis is poor, with an overall survival of 19–21 months (Suzuki & Nakamura, 1999; Oshimi *et al*, 2005).

"Precursor NK-lymphoblastic leukaemia/lymphoma"

Precursor NK-cell ALL

Many cases of $CD56^+$ ALL without T and B cell-lineage characteristics have been reported previously, and our NK-cell Tumour Study Group proposed classification of these ill-defined ALLs as precursor NK-cell ALL (Oshimi, 2000, 2003; Oshimi *et al*, 2005; Suzuki *et al*, 2005). Our original diagnostic criteria included the presence of 30% or more lymphoblasts in the bone marrow or peripheral blood, $CD56^+CD3^-$, B-cell antigen $(CD19 \text{ and } CD20)^-$ and myeloid antigen $(CD13 \text{ and } CD33)^-$ phenotypes, and the germ-line configuration of *TRB@* and *IGH@*. Based on the above criteria, we have collected 27 cases over 5 years (Suzuki *et al*, 2005). This is a rare disease that develops both in children and in adults, with a median age of 55 years. Lymphadenopathy, hepatosplenomegaly, and skin lesions are common. The prognosis is poor, with an overall survival of 20 months.

Blastic NK-cell lymphoma

Blastic NK-cell lymphoma was thought to develop from committed NK precursor cells. Our diagnostic criteria (Oshimi, 2000, 2003; Oshimi *et al*, 2005; Suzuki *et al*, 2005) included mass formation by a proliferation of lymphoblastoid cells with immunophenotypes of $CD56^+CD3^-CD4^{+/-}$, B-cell antigens $(CD19 \text{ and } CD20)^-$ and myeloid antigens $(CD13 \text{ and } CD33)^-$, and with the genotype of the germ-line configuration *TRB@* and *IGH@*. CD4 was not required as a mandatory marker, because many $CD4^-$ cases have been reported (Karube *et al*, 2003). Patients with >30% tumour cells in the bone marrow or peripheral blood were categorized as having precursor NK-cell ALL, and the others as blastic NK-cell lymphoma. This is a rare disease, presenting mainly with skin lesions, and occasional leukaemic change. Prognosis is poor, with an overall survival of 17 months (Suzuki *et al*, 2005).

Proposal of a new clinical entity

From our subsequent studies, however, the above two diseases were found to be indistinguishable in terms of their clinical features, except for the presence or absence of hepatosplenomegaly and the haematological data including tumour cells in the bone marrow or peripheral blood (Suzuki *et al*, 2005). Accordingly, I would like to propose the name 'precursor NK-lymphoblastic leukaemia/lymphoma' as a collective classification, based on the WHO nomenclature of precursor T- and B-lymphoblastic leukaemia/lymphoma. Precursor NK-lymphoblastic leukaemia/lymphoma conceivably arises from stage 3 iNK cells, or from a putative stage between stage 3 and stage 4 because precursor NK-lymphoblastic leukaemia/lymphoma cells lack the CD33 antigen. Recent reports from France indicate that $CD4^+CD56^+$ blastic NK-cell lymphoma or haematodermic neoplasm (recently proposed as pDC leukaemia/lymphoma) arises from pDC precursors, but not from NK precursors (reviewed by Garnache-Ottou *et al*, 2007). However, because normal pDC are mostly $CD56^-$, $CD4^+CD56^-$ pDC leukaemia/lymphoma will also be present. Considering that pDC lymphoblastic leukaemia/lymphoma is mainly found in the non-T, non-B, $CD4^+CD56^+$ subset, precursor NK-lymphoblastic leukaemia/lymphoma should be found mainly in the non-T, non-B, $CD4^-CD56^+$ subset. However, because the CD4 antigen seems to be expressed in some T/NK common progenitors (Plum *et al*, 1999), $CD4^+CD56^+$ true NK-cell tumours may be present. Within non-T, non-B, $CD4^-CD56^-$ ALL, precursor NK-lymphoblastic leukaemia/lymphoma may also be found because stage 3 committed iNK cells are partly $CD56^-$ (Fig 1). Taken together, the lineages of non-T, non-B, lymphoblastic leukaemias/lymphomas are not well identified, and markers exclusive to certain lineages should be explored. The presence of CD94 1A transcripts suggests precursor NK-cell lineage, but not T-cell lineage (Lin *et al*, 2005), and this may improve the current classification of NK cell- and T cell-lineage tumours. The NK-cell lineage can also be confirmed when purified tumour cells in culture develop NK-lineage features *in vitro*. EBV is not detected in the tumour cells.

Aggressive NK-cell leukaemia

Aggressive NK-cell leukaemia was originally reported by Fernandez *et al* (1986) and Koizumi *et al* (1986), and further described by Imamura *et al* (1990), Song *et al* (2002) and Suzuki *et al* (2004).

Clinical features

Younger patients are mainly affected, and fever, hepatosplenomegaly and lymphadenopathy are common. The clinical course is rapidly progressive, and the prognosis is poor with an overall survival of 2 months (Suzuki *et al*, 2004). Liver dysfunction, disseminated intravascular coagulation, and

haemophagocytic syndrome are often seen during the course of the disease, particularly at the terminal stage.

Diagnosis

This disease is characterized by the presence of NK cells of slightly immature-looking morphology mainly in the peripheral blood, bone marrow, liver and spleen, and by a rapidly progressive clinical course with poor outcome. Diagnosis is made when the following findings are fulfilled (Oshimi, 2000, 2003; Suzuki *et al*, 2004; Oshimi *et al*, 2005): (i) morphologically, slightly immature-looking large lymphocytes, with broad, pale cytoplasm and azurophilic granules, and somewhat fine nuclear chromatins and occasional nucleoli, are present in the peripheral blood and/or bone marrow; (ii) immunophenotypically, these lymphocytes are surface (s) $CD3^-$ and cytoplasmic (c) $CD3\epsilon^{+/-}$ and $CD56^+CD16^{-/+}CD57^-$; and (iii) genetically, they have germ-line configuration of *TRB@* and *IGH@*. The NK cells appear slightly immature in their morphology, but the presence of the CD94 antigen indicates their mature cell origin (Mori *et al*, 2001).

At presentation, this disease is sometimes indistinguishable from chronic NK-cell lymphocytosis, but from our experience, the presence of conspicuous nucleoli in NK cells strongly supports the diagnosis of aggressive NK-cell leukaemia. Also, age <40 years old, fever, lymph node swelling, and hepatosplenomegaly predict this diagnosis (Oshimi *et al*, 1993). Extranodal NK/T-cell lymphoma may also present as, or eventually develop, leukaemic changes (Soler *et al*, 1994), and the distinction between aggressive NK-cell leukaemia and extranodal NK/T-cell lymphoma is sometimes difficult unless a nasal lesion is found. The incidence of skin involvement, however, is significantly higher for extranodal NK-cell lymphoma (Oshimi *et al*, 2005). An array-based comparative genomic hybridization analysis demonstrated clear genetic differences between aggressive NK-cell leukaemia and extranodal NK/T-cell lymphoma, suggesting that these are two distinct diseases (Nakashima *et al*, 2005).

Pathogenesis

In most patients, clonal EBV is found in tumour cells, and EBV is considered to be the aetiological agent (Kawa-Ha *et al*, 1989; Siu *et al*, 2002). However, little is known of how infection with EBV might trigger clonal growth of NK cells. Various genes that possibly contribute to tumourigenesis have been identified in EBV-positive extranodal NK/T-cell lymphoma, but a role of these genes in aggressive NK-cell leukaemia has not been identified except for high expression of the MDM2 protein (Sugimoto *et al*, 2002). A retroviral screening method failed to isolate tumour-promoting genes (Choi *et al*, 2005). Various chromosomal abnormalities have been reported, and the finding of the same chromosomal abnormality involving del(6q) in aggressive NK-cell leukaemia and in extranodal NK/T-cell lymphoma provides a biological link between these two diseases (Wong *et al*, 1997; Ohshima *et al*, 2002). Using comparative genomic hybridization, recurrent regions of gain and loss of chromosomes have been identified, suggesting that leukaemogenesis in aggressive NK-cell leukaemia is associated with multiple steps of oncogene activation and suppressor oncogene loss (Siu *et al*, 1999, 2000; Nakashima *et al*, 2005).

According to our previous review (Oshimi *et al*, 1993), CD16 was absent in 5/12 cases, natural cytotoxicity was absent in 3/13 cases, and ADCC was absent in 6/11 cases, implying that some cases may arise from stage 4 mNK cells and some from stage 5 mNK cells. Production of various cytokines from the tumour cells (see below) further supports their stage 4 mNK origin, and it will be interesting to determine whether clinical features depend on the stage of the cells from which the tumours originate.

Liver dysfunction is probably caused by Fas ligand-bearing NK cells that induce apoptotic cell death of Fas-expressing hepatocytes (Tani *et al*, 1999). Among various chemokine receptors tested, CXCR1 and CCR5 are simultaneously expressed on aggressive NK-cell leukaemia cells, and the leukaemic cells showed enhanced chemotaxis towards the chemokines of these receptors (Makishima *et al*, 2005). Further, the serum levels of the corresponding chemokines interleukin (IL)-8, RANTES (regulated on activation, normal T-cell expressed and secreted), macrophage inflammatory protein (MIP)-1α and MIP-1β are significantly elevated, and these chemokines are produced by leukaemic cells and hepatocytes. Interaction between these chemokines produced from hepatocytes and chemokine receptors on leukaemic cells may play an important role in the transmigration of tumour cells to hepatocytes (Makishima *et al*, 2007). Haemophagocytic syndrome may be because of the upregulation of TNF-α by EBV-infected tumour cells, and TNF-α in combination with IFN-γ and other cytokines activates macrophages (Lay *et al*, 1997). IFN-γ also prevents apoptosis of tumour cells in an autocrine fashion (Mizuno *et al*, 1999). Soluble Fas ligand levels in the serum may be a useful indicator for evaluating disease activity (Kato *et al*, 1998). Thus, various types of cytokines produced by leukaemic cells are closely related to the clinical features observed in this disease.

Treatment

Because of its rare incidence, there is no known treatment strategy. The disease is refractory to chemotherapy and exhibits a relentless progressive course with a poor outcome. As in extranodal NK/T-cell lymphoma, this poor outcome may be partially explained by the presence of P-glycoprotein (P-gp), a multidrug resistance gene-encoded protein on the cell membrane, that extrudes various cytotoxic agents, such as vinca alkaloids and anthracyclines (Yamamoto *et al*, 1993; Yamaguchi *et al*, 1995; Egashira *et al*, 1999), and combination chemotherapies using P-gp-unrelated drugs are to be expected. Allogeneic haematopoietic stem cell transplantation (HSCT) has been tried with only a few successful cases being reported.

Extranodal NK/T-cell lymphoma

In the new WHO classification, this disease is called extranodal NK/T-cell lymphoma, nasal type. It is called 'NK/T' rather than simply 'NK' because although most cases have genuine NK-cell neoplasm, some have cytotoxic T-cell neoplasm (Suzumiya *et al*, 1994). The primary site of involvement is the nasal cavity, but sometimes identical neoplasms primarily develop in the extranasal sites. The term 'nasal-type' describes the disease that arises in the nasal cavity and also in the extranasal sites. However, this term is confusing, and a term more clearly understandable should be applied to combine both conditions. In this review, the term 'extranodal NK-cell lymphoma' is used when referring to NK-cell tumours alone, whereas the term 'extranodal NK/T-cell lymphoma' is used when referring to both NK-cell and T-cell tumours.

Clinical features

Extranodal NK/T-cell lymphoma is characterized by nasal and extranasal presentation, as well as an aggressive clinical course. This lymphoma is rare in the Western countries, but is relatively common in East Asia and Latin America. Its frequency among all malignant lymphomas is around 3% in Japan, 6% in Hong Kong, 7% in Taiwan and 9% in Korea. It develops in middle-aged persons, and males are more often affected than females. The nose and paranasal area including the upper aerodigestive tract are involved in >80% of cases, and less often the skin, intestinal tract and various other organs are involved at presentation. The ratio of patients with limited stages (clinical stage I or II) *versus* those with advanced stages (clinical stage III or IV) is 7:3 for lymphomas of nasal origin, and 4:6 for lymphomas of extranasal origin (Oshimi *et al*, 2005).

Patients with nasal lesions present with symptoms of mass, nasal obstruction or epistaxis. The tumour is locally invasive and may infiltrate the surrounding tissues and organs, such as the orbits, nasopharynx, oropharynx and palate, or generate extensive midfacial destructive lesions (so-called lethal midline granuloma). The skin is commonly involved in the form of nodules, often with ulceration. Intestinal lesions often manifest as perforation or bleeding. With dissemination of the disease, fever, malaise and weight loss can develop, and bone marrow and blood involvement can occur. The central nervous system is affected in 7% of patients at presentation (Oshimi *et al*, 2005) by direct invasion from nasal lymphoma or by metastasis. CD56 expressed on NK cells is a neural adhesion molecule, and mediates adhesion through homophilic binding. This adhesion molecule is also expressed in nerve tissues. NK-cell malignancies could therefore have an affinity for nerve tissues. Similarly to aggressive NK-cell leukaemia, extranodal NK/T-cell lymphoma is frequently associated with haemophagocytic syndrome, typically at the terminal phase of the disease (Takahashi *et al*, 2001), and this is probably induced by the same mechanism described above for aggressive NK-cell leukaemia (Lay *et al*, 1997).

Diagnosis

The diagnosis of extranodal NK-cell lymphoma is made when the following criteria are fulfilled. (1) On histology and morphology, a diffuse, non-adhesive proliferation of lymphoid cells is found with frequent association of tissue necrosis and coagulation. Sometimes a mixture of inflammatory cells is present. Infiltration of the tumour cells is frequently perivascular (angiocentric) and angiodestructive, and the cytological spectrum of the tumour cells is broad, but the cells are medium-sized in most cases. Imprint smears can disclose the presence of cytoplasmic azurophilic granules. (2) Immunophenotype is as follows; $CD45(LCA)^{+}sCD3^{-}cCD3\epsilon^{+}CD56^{+}$, myeloid antigens^{-}, and B-cell antigens^{-} (Suzumiya *et al*, 1994). Cytotoxic granule-associated proteins, granzyme B, TIA-1 and perforin, are positive. (3) Upon gene rearrangement analysis, *TRB@* and *IGH@* are in the germ-line configuration. (4) EBV in tumour cells is almost always detectable. Because of its predilection for vessels and other implicated factors (Teruya-Feldstein *et al*, 1997), vascular occlusion with massive necrosis of the tissue is often one of the presenting features, and it is sometimes difficult to make a diagnosis, even with repeated biopsies. Detection of $CD56^{+}$ cells or EBV-positive cells in paraffin-embedded specimens is important for diagnosis because these findings are rarely observed in lymphocytes residing in normal or inflammatory nasal mucosa or adjacent tissue.

Among patients with nasal lymphoma, 3/4 patients have NK- or T-cell lymphoma, and the remaining have B-cell lymphoma. The incidence of T-cell lymphoma seems to be much lower than that of NK-cell lymphoma, but its true incidence is not known because the distinction between NK and T lineage is sometimes difficult. As shown in Table I,

Table I. Distinction between mature T and NK cells.

	T cells	NK cells
Surface markers		
CD2	+	+
CD3	+	−
CD5	+/−	−
CD7	+	+
CD16	−/+	+/−
CD56	−/+	+/−
TCR	+	−
Cytoplasmic CD3ϵ	+	−/+
TCR gene rearrangement	+	−
LGL morphology	−/+	+
Natural cytotoxicity	−/+	+
NK receptors	−/+	+

TCR, T-cell receptor; LGL, large granular lymphocytes.

sCD3 is positive in T cells, and negative in NK cells, but cCD3ε is positive both in T and activated, but not resting, NK cells (Lanier *et al*, 1992). Because it is difficult to discriminate the sCD3 antigen from the cCD3ε antigen in paraffin-embedded specimens, distinction between NK and T lineages is difficult when only paraffin-embedded specimens are available. CD5 positivity, however, strongly suggests T-cell lineage (Emile *et al*, 1996). The demonstration of sCD3 by flow cytometry and monoclonal TCR gene bands by Southern blot analysis is unique to T cells, and these two procedures should be routinely employed. LGL morphology, as demonstrated by Giemsa staining of imprint smears, also suggests NK-cell lineage, but some T-cell lymphomas also have such morphology. Although it is unknown whether vigorous efforts to differentiate NK from T lineage are clinically important or not in terms of treatment strategies or prognoses, the effort should still be made to get sufficient amounts of biopsy material to delineate the clinical features of each T-cell and NK-cell nasal lymphoma.

Pathogenesis

Extranodal NK/T-cell lymphoma is almost always associated with EBV (Harabuchi *et al*, 1990). Using *in situ* hybridization techniques, EBV-encoded small RNA can be found in the tumour cells, and Southern blot analysis can detect monoclonal proliferation of EBV. These findings indicate that EBV infection has been established at the early stage of tumourigenesis and strongly suggests its aetiologic role. Our experiments have clearly shown that EBV easily infects NK cells (Isobe *et al*, 2004). Interestingly, this infection occurs in the absence of CD21 antigen, the EBV receptor, on the NK-cell surface. The majority of Japanese patients have subtype A EBV infection with a 30-bp deletion in the latent membrane protein (LMP)-1 gene (Suzumiya *et al*, 1999). Sequence variations of the EBV LMP-1 gene and amino acid changes at HLA-restricted cytotoxic T-cell epitopes may be associated with an increase in tumourigenicity and with a decrease in immune recognition (Nagamine *et al*, 2007).

In addition to conventional chromosomal analysis, various procedures including comparative genomic hybridization, loss of heterozygosity, and fluorescence *in-situ* hybridization have been applied to demonstrate genetic abnormalities. The results indicate that complex chromosomal abnormalities are often seen (Wong *et al*, 1997), and a frequent deletion at 6q has been found (Tien *et al*, 1997; Wong *et al*, 1997). DNA gains and losses are also frequent (Siu *et al*, 1999, 2000; Nakashima *et al*, 2005). Various types of genes are altered, and these include *TP53, TP73CDKN2A, CDKN2B, MDM2, FAS* and *KIT* (reviewed by Suzuki, 2005). Further, a recent report describes alterations in *ATR* (Liu *et al*, 2006). The T-helper cell, type 1 (Th1) cytokine transcription factor T-bet, which also influences the development of NK cells, is amplified and specifically expressed in extranodal NK/T-cell lymphoma (Ye *et al*, 2007). The relationship between EBV infection and chromosomal and genetic abnormalities remains to be clarified. When lifestyle and environmental factors were assessed, exposure to pesticide and solvents was found to be a risk factor (Xu *et al*, 2006).

According to our previously studied case, CD16 was dim and CD56 was bright, with strong ADCC and natural cytotoxicity (Kaneko *et al*, 1995). Thus the phenotype indicates a stage 4 mNK cell origin, whereas the potent cytotoxicity indicates a stage 5 mNK origin. Other reports indicate that CD16 is usually negative, and, to the best of this authors' knowledge, the level of cytotoxicity has not been reported, suggesting that most tumours originate from stage 4 mNK cells. Production of various types of cytokines, such as IFN-γ and TNF-α, from the tumour cells also supports stage 4 mNK origin. In normal NK-cell development, stage 4 mNK cells outnumber stage 5 mNK cells, and stage 4 mNK cells are present mainly in the lymph nodes and tonsils (Ferlazzo *et al*, 2004). Extranodal NK-cell lymphoma, however, develops mainly in sites other than lymph nodes and tonsils. It is unknown why this lymphoma originates from the extranodal sites. The presence of the activated antigens HLA-DR, CD71 and cCD3ε supports that the lymphoma cells are in the activated state (Kaneko *et al*, 1995).

Staging and prognosis

The Ann Arbor staging classification system was originally developed for Hodgkin lymphoma, a disease primarily developing from and spreading to the lymph nodes. However, extranodal NK/T-cell lymphoma primarily develops from and spreads to the extranodal sites, and this staging system does not clearly predict survival differences. Also, the International Prognostic Index for aggressive B-cell lymphomas seems to be inadequate to predict them. The following parameters have individually been reported to indicate poor prognosis; advanced stage, local tumour invasiveness, bone marrow involvement, extra-upper aerodigestive tract origin, high proliferation index, high plasma EBV level, low expression of CD94 transcripts, expression of cutaneous lymphocyte antigen, absence of granzyme B inhibitor PI9, *TP53* missense mutations, and high serum level of the nm23-H1 protein. However, a new staging system that precisely predicts the prognosis is anticipated. Recently, Lee *et al* (2006a) proposed a prognostic model based on B symptoms, stage, lactate dehydrogenase level, and regional lymph node involvement, allowing a better prognostic discrimination when compared with the International Prognostic Index.

Procedures for the pretreatment evaluation of lymphoma include computed tomography scanning and ^{67}gallium scintigraphy. Many studies have recently demonstrated the utility of ^{18}fluoro-2-deoxyglucose positron emission tomography (FDG-PET) for staging and the therapeutic response assessment of lymphoma, and a recent study clearly indicated that pretreatment FDG-PET scans are also highly useful for evaluating extranodal NK/T-cell lymphoma (Kako *et al*, 2007a).

Treatment

Seventy percent or more of patients with extranodal NK/T-cell lymphoma present with localized disease. In localized diffuse large B-cell lymphoma, three courses of CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) followed by radiation therapy give excellent results. However, in localized extranodal NK/T-cell lymphoma, outcomes using this protocol seem to be discouraging. Response of the primary tumours to such chemotherapy is transient if present, and early local relapse or metastasis is common (Kwong, 2005; Oshimi *et al*, 2005; Suzuki, 2005). Compared with anthracycline-based chemotherapies, first-line local radiotherapy of a total dose of 50 Gy with generous margins is effective (Isobe *et al*, 2006), but radiotherapy alone is not sufficient, with only 30–40% long-term survival, possibly because of the presence of unidentified metastatic legions in some patients. The addition of anthracycline-based chemotherapy after radiation, however, does not appear to significantly modify the survival of patients (Li *et al*, 2006), conceivably because of early dissemination of the disease before the beginning of chemotherapy, and because of the presence of P-gp in NK cells as discussed above. In Japan, therefore, a nation-wide, prospective phase I/II trial (JCOG0211) consisting of concurrent chemoradiotherapy for localized nasal NK/T-cell lymphoma has been conducted (Yamaguchi *et al*, 2005). Local radiotherapy (50 Gy) was given with precise computed tomography-based planning to improve the appropriate dose distribution of the target volumes, and three courses of DeVIC (dexamethasone, etoposide, ifosfamide and carboplatin) were administered. This trial was based on an observation made by Yamaguchi *et al* (2001), who reported a good clinical outcome in combination chemotherapy using DeVIC and concurrent local radiotherapy for nasal NK-cell lymphoma. This success may be partly because of the choice of DeVIC combination chemotherapy, because the agents included in this regimen are unrelated to P-gp, except for etoposide. Another group (Lee *et al*, 2006b) also reported good results using P-gp-unrelated agents, and therefore avoiding P-gp-related agents seems to show promise.

The prognosis of the advanced disease is extremely poor. Conventional chemotherapy regimens, such as CHOP or other anthracycline-based regimens, are discouraging with an overall 5-year survival rate of <20%. When P-gp-unrelated agents are mainly used, the prognosis seems to be better. Aviles *et al* (2003) treated patients with advanced disease with CMED (cyclophosphamide, methotrexate, etoposide and dexamethasone) chemotherapy and radiotherapy: patients were given three courses of CMED, followed by 55-Gy irradiation mainly to the face and three additional courses of CMED therapy; the complete response rate and 5-year overall survival were 65%.

In vitro studies and clinical experiences indicate various types of chemotherapeutic agents to be effective for extranodal NK/T-cell lymphoma. Similarly to ALL, several cases of nasal NK-cell lymphoma show low asparagine synthetase activity and high *in vitro* sensitivity to L-asparaginase (Ando *et al*, 2005), and successful treatment has been reported for relapsed cases of extranodal NK/T-cell lymphoma by L-asparaginase alone. For combination chemotherapy using L-asparaginase, a Chinese group reported a good result for CHOP failures (Yong *et al*, 2003). In recent years, data have accumulated concerning the efficacy of etoposide for NK/T-cell malignancies. An *in vitro* anti-tumour effect of etoposide, in combination with cyclosporin, has been shown for extranodal NK/T-cell lymphoma (Uno *et al*, 2001). Good clinical results for etoposide-containing chemotherapy without anthracycline have been reported (Lee *et al*, 2006b). Etoposide is a key drug for pediatric EBV-related haemophagocytic syndrome (Imashuku, 2000), and the addition of etoposide will also be good for haemophagocytic syndrome often associated with extranodal NK/T-cell lymphoma. Methotrexate and ifosfamide also seem to be effective because they are P-gp-unrelated agents, and have been included in treatment regimens for NK/T-cell lymphomas. Based on these observations, and paying special attention to the pharmacokinetics of sequential or simultaneous administration, our NK-cell Tumour Study Group has conducted a newly designed, prospective phase I study of 'SMILE' for advanced, refractory, or relapsed nasal-type NK/T-cell lymphoma. The SMILE protocol consisted of steroid (dexamethasone), methotrexate, ifosfamide, L-asparaginase and etoposide, and gave safe and promising results (Kwong *et al*, 2007). When the central nervous system is involved, it is difficult to eradicate tumour cells. Although the standard treatment procedures are not established, systemic administration of high-dose methotrexate and intrathecal administration of steroids may be helpful.

Liang *et al* (1997) reported successful outcomes in two of three patients who received autologous HSCT. Autologous HSCT performed during the inactive state tends to provide a better prognosis than that carried out during the active state (Au *et al*, 2003; Kim *et al*, 2006). However, it is unknown whether autologous HSCT is necessary or not during the inactive state, and a randomized trial will be required. Patients who receive autologous HSCT in advanced disease may also have a favourable outcome when compared with those who do not receive it (Kim *et al*, 2006). Many patients underwent allogeneic HSCT during active disease and achieved long-term relapse-free survival (Murashige *et al*, 2005; Suzuki *et al*, 2006). One of the explanations for their success is a graft-versus-lymphoma effect (Kako *et al*, 2007b). However, the optimal timing for allogeneic HSCT remains undetermined. When effective chemotherapeutic regimens are developed, many patients will be able to receive HSCT during the inactive state, and this will give further success.

In extranodal NK/T-cell lymphoma, EBV is almost always found in the tumour cells. Adoptive transfer of EBV-specific cytotoxic T lymphocytes was therefore attempted, with two of three patients being induced to a stable disease (Cho *et al*, 2006). Monoclonal antibodies against CD45, CD52 and CD56 have been tried or are planned.

Chronic NK-cell lymphocytosis

Disorders with granular lymphocyte proliferation

There are patients who exhibit expansion of granular lymphocytes in the peripheral blood. These lymphocytes are either T cells or NK cells depending on their surface phenotype. The names granular lymphocyte-proliferative disorders (GLPD) (Oshimi, 1988; Oshimi *et al*, 1993), lymphoproliferative disease of granular lymphocytes (Semenzato *et al*, 1987) and LGL leukaemia (Loughran & Starkebaum, 1987) have been coined to describe these disorders. T-cell proliferation is usually monoclonal and NK-cell proliferation is probably reactive in most cases, with rare cases exhibiting an aggressive clinical course. NK-cell proliferation with an indolent clinical course is called chronic NK-cell lymphocytosis, and a disease with an aggressive clinical course is called aggressive NK-cell leukaemia. When monoclonal T cells are expanded, the term 'T-cell LGL leukaemia' is used in the WHO classification. However, from my personal observation, the diameter of these T cells is usually less than double of that of erythrocytes and smaller than that of NK-lineage LGL, thus indicating that they are not 'large' lymphocytes. Further, most cases exhibit an indolent clinical course without disease progression, and some cases regress spontaneously. Thus it is inappropriate to call this 'leukaemia'; it is rather a 'T-cell clonopathy of benign or undetermined significance', and I prefer to call these disorders GLPD collectively.

Clinical features and diagnosis of chronic NK-cell lymphocytosis

Chronic NK-cell lymphocytosis is characterized by the chronic expansion of mature-looking NK cells in the peripheral blood (Oshimi *et al*, 1993, 2005; Tefferi *et al*, 1994; Oshimi, 2003). Diagnosis can be made when $0{\cdot}6 \times 10^9$/l or more of $sCD3^-CD56^{+/-}CD16^+$ NK cells are present in the peripheral blood for at least 6 months. Because expanding NK cells are almost always positive for CD16 with potent natural cytotoxicity and ADCC (Oshimi *et al*, 1993), this disorder is probably of stage 5 mNK origin. Clinically, most patients present with a chronic indolent course and absence of any symptoms, and the NK cells are considered to be reactively proliferated (Nash *et al*, 1993), although phenotypic deviation of NK receptors is found (Zambello *et al*, 1993). The absence of symptoms may be explained by decreased production of cytokines from stage 5 mNK cells compared with stage 4 mNK cells.

Pathogenesis of chronic NK-cell lymphocytosis

Considering the role of NK cells in innate immunity, some aetiologic agents may stimulate and expand a subset of the NK-cell population. Indeed, the possible association between chronic viral infections and skewed NK-cell proliferation has been described (Zambello *et al*, 1995; Loughran *et al*, 1997), but the issue of whether these events stimulate a clonal process *per se* and eventually progress toward malignant disease deserves further study.

Clinical course of chronic NK-cell lymphocytosis

In chronic NK-cell lymphocytosis, the number of NK cells is stable without treatment or it sometimes regresses spontaneously. Rare cases transform to aggressive NK-cell leukaemia, and patients with EBV-positive NK cells tend to evolve. These EBV-positive patients usually have chronic active EBV infection, hypersensitivity to mosquito bites or hydroa vacciniforme, and they should be carefully followed for the emergence of clonal NK cells (Kawa-Ha *et al*, 1989; Kawa, 2003; Oshimi *et al*, 2005). The clonality of EBV-positive NK cells can be examined by Southern blotting using a probe recognizing the EBV terminal repeat. A monoclonal increase in NK cells is noted in a substantial number of such patients, thus indicating that EBV-associated chronic NK-cell lymphocytosis may develop clonal NK-cell proliferation with a likelihood of eventual progression of the disease to aggressive NK-cell leukaemia or extranodal NK-cell lymphoma (Kawa-Ha *et al*, 1989; Kawa, 2003; Oshimi *et al*, 2005).

Future prospects

To make clear the characteristics of NK-lineage tumours, an understanding of the developmental pathway of normal NK cells is crucial. Because T- and NK-lineage cells are closely related in their developmental pathway, tumours of T- and NK-lineage cells are difficult to differentiate, and an antigen exclusively expressed in NK cells, but not in T cells, that can be detected in paraffin-embedded specimens should be explored. The differences between cytotoxic T and NK cells are not fully understood, and DNA profiling using purified normal and abnormally expanded cytotoxic T and NK cells will help to resolve this, and also help to understand the differences in clinical behaviours between cytotoxic T- and NK-derived tumours. The mechanism by which EBV transforms NK cells is not fully understood, and the elucidation of this mechanism may lead to a new molecular therapeutic target. Also, the reasons why NK-cell tumours are resistant to chemotherapy are not fully understood, and the molecules responsible for proapoptosis and antiapoptosis should be characterized as well as drug-resistance genes. Finally, new therapies targeting abnormal molecules should be extensively explored.

Acknowledgements

I thank all the members of our NK-cell Tumour Study Group, particularly Drs Ritsuro Suzuki, Motoko Yamaguchi, Junji Suzumiya, Koji Izutsu, Keisei Kawa, Shigeo Nakamura, Koichi Ohshima, Yok-Lam Kwong, Won Seog Kim, Yasuhiko Kano, Fumihiro Ishida, Takayuki Okamura, Kengo Takeuchi and Yasushi Isobe for their help and advice. This work was supported in part by grants from the Ministry of Health,

Labour and Welfare of the Japanese Government, and by Kirin Brewery Co. Ltd.

References

Ando, M., Sugimoto, K., Kitoh, T., Sasaki, M., Mukai, K., Ando, J., Egashira, M., Schuster, S.M. & Oshimi, K. (2005) Selective apoptosis of natural killer-cell tumours by L-asparaginase. *British Journal of Haematology*, **130,** 860–868.

Au, W.Y., Lie, A.K.W., Liang, R., Kwong, Y.-L., Yau, C.-C., Cheung, M.M.C., Ngan, K.-C., Lau, W.-H., Wong, K.-H., Yiu, H.-Y., Cheng, H.-C., Au, K.-H. & Chan, J.K.C. (2003) Autologous stem cell transplantation for nasal NK/T-cell lymphoma: a progress report on its value. *Annals of Oncology*, **14,** 1673–1676.

Aviles, A., Neri, N., Fernandez, R., Calva, A., Huerta-Guzman, J. & Nambo, M.J. (2003) Nasal NK/T-cell lymphoma with disseminated disease treated with aggressive combined therapy. *Medical Oncology*, **20,** 13–17.

Chan, J.K.C. (1997) CD56-positive putative natural killer (NK) cell lymphoma: nasal, nasal-type, blastoid, and leukaemic forms. *Advances in Anatomic Pathology*, **4,** 163–172.

Cheung, M.M., Chan, J.K. & Wong, K.F. (2003) Natural killer cell neoplasms: a distinctive group of highly aggressive lymphomas/leukaemias. *Seminars in Haematology*, **40,** 221–232.

Cho, H.I., Hong, Y.S., Lee, M.A., Kim, E.K., Yoon, S.H., Kim, C.C. & Kim, T.G. (2006) Adoptive transfer of Epstein-Barr virus-specific cytotoxic T-lymphocytes for the treatment of angiocentric lymphomas. *International Journal of Haematology*, **83,** 66–73.

Choi, Y.L., Moriuchi, R., Osawa, M., Iwama, A., Makishima, H., Wada, T., Kisanuki, H., Kaneda, R., Ota, J., Koinuma, K., Ishikawa, M., Takada, S., Yamashita, Y., Oshimi, K. & Mano, H. (2005) Retroviral expression screening of oncogenes in natural killer cell leukaemia. *Leukaemia Research*, **29,** 943–949.

Cooper, M.A., Fehniger, T.A., Turner, S.C., Chen, K.S., Ghaheri, B.A., Ghayur, T., Carson, W.E. & Caligiuri, M.A. (2001) Human natural killer cells: a unique innate immunoregulatory role for the $CD56^{bright}$ subset. *Blood*, **97,** 3146–3151.

Egashira, M., Kawamata, N., Sugimoto, K., Kaneko, T. & Oshimi, K. (1999) P-glycoprotein expression on normal and abnormally expanded natural killer cells and inhibition of P-glycoprotein function by cyclosporine A and its analogue, PSC833. *Blood*, **93,** 599–606.

Emile, J.-F., Boulland, M.-L., Haioun, C., Kanavaros, P., Petrella, T., Delfau-Larue, M.-H., Bensussan, A., Farcet, J.-P. & Gaulard, P. (1996) $CD5^{-}$ $CD56^{+}$ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphoma. *Blood*, **87,** 1466–1473.

Fehniger, T.A., Cooper, M.A., Nuovo, G.J., Cella, M., Facchetti, F., Colonna, M. & Caligiuri, M.A. (2003) $CD56^{bright}$ natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood*, **101,** 3052–3057.

Ferlazzo, G., Thomas, D., Lin, S.-L., Goodman, K., Morandi, B., Muller, W.A., Moretta, A. & Munz, C. (2004) The abundant NK cells in human secondary lymphoid tissues require activation to express killer cell Ig-like receptors and become cytolytic. *Journal of Immunology*, **172,** 1455–1462.

Fernandez, L.A., Pope, B., Lee, C. & Zayed, E. (1986) Aggressive natural killer cell leukaemia in an adult with establishment of an NK cell line. *Blood*, **67,** 925–930.

Freud, A.G. & Caligiuri, M.A. (2006) Human natural killer cell development. *Immunological Reviews*, **214,** 56–72.

Freud, A.G., Yokoyama, A., Becknell, B., Lee, M.T., Mao, H.C., Ferketich, A.K. & Caligiuri, M.A. (2006) Evidence for discrete stages of human natural killer cell differentiation *in vivo*. *Journal of Experimental Medicine*, **203,** 1033–1043.

Garnache-Ottou, F., Feuillard, J. & Saas, P. (2007) Plasmacytoid dendritic cell leukaemia/lymphoma: toward a well defined entity? *British Journal of Haematology*, **136,** 539–548.

Harabuchi, Y., Yamanaka, N., Kataura, A., Imai, S., Kinoshita, T., Mizuno, F. & Osato, T. (1990) Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. *Lancet*, **335,** 128–130.

Hercend, T. & Schmidt, R.E. (1988) Characteristics and uses of natural killer cells. *Immunologyl Today*, **9,** 291–293.

Imamura, N., Kusunoki, Y., Kawa-Ha, K., Yumura, K., Hara, J., Oda, K., Abe, K., Dohy, H., Inada, T., Kajihara, H. & Kuramoto, A. (1990) Aggressive natural killer cell leukaemia/lymphoma: report of four cases and review of the literature. Possible existence of a new clinical entity originating from the third lineage of lymphoid cells. *British Journal of Haematology*, **75,** 49–59.

Imashuku, S. (2000) Advances in the management of haemophagocytic lymphohistiocytosis. *International Journal of Haematology*, **72,** 1–11.

Isobe, Y., Sugimoto, K., Yang, L., Tamayose, K., Egashira, M., Kaneko, T., Takada, K. & Oshimi, K. (2004) Epstein-Barr virus infection of human natural-killer cell lines and peripheral blood natural killer cells. *Cancer Research*, **64,** 2167–2174.

Isobe, K., Uno, T., Tamura, J., Kawakami, H., Ueno, N., Wakita, H., Okada, J., Itami, J. & Ito, H. (2006) Extranodal natural killer/T-cell lymphoma, nasal type: the significance of radiotherapeutic parameters. *Cancer*, **106,** 609–615.

Jaffe, E.S. (1996) Classification of natural killer (NK) cell and NK-like T-cell malignancies. *Blood*, **87,** 1207–1210.

Kako, S., Izutsu, K., Ohta, Y., Minatani, Y., Sugaya, M., Momose, T., Ohtomo, K., Kanda, Y., Chiba, S. & Kurokawa, M.. (2007a) FDG-PET in T-cell and NK-cell neoplasms. *Annals of Oncology*, in press.

Kako, S., Izutsu, K., Oshima, K., Sato, H., Kanda. Y., Motokura.T., Chiba, S. & Kurokawa, M.. (2007b) Regression of the tumor after withdrawal of cyclosporine in relapsed extranodal natural killer/T cell lymphoma following allogeneic hematopoietic stem cell transplantation. *American Journal of Haematology*, in press.

Kaneko, T., Fukuda, J., Yoshihara, T., Zheng, H., Mori, S., Mizoguchi, H. & Oshimi, K. (1995) Nasal natural killer (NK) cell lymphoma: report of a case with activated NK cells containing Epstein-Barr virus and expressing CD21 antigen, and comparative studies of their phenotype and cytotoxicity with normal NK cells. *British Journal of Haematology*, **91,** 355–361.

Karube, K., Ohshima, K., Tsuchiya, T., Yamaguchi, T., Suefuji, H., Suzumiya, J., Harada, M. & Kikuchi, M. (2003) Non-B, non-T neoplasms with lymphoblast morphology: further clarification and classification. *American Journal of Surgical Pathology*, **27,** 1366–1374.

Kato, K., Ohshima, K., Ishihara, S., Anzai, K., Suzumiya, J. & Kikuchi, M. (1998) Elevated serum soluble Fas ligand in natural killer cell proliferative disorders. *British Journal of Haematology*, **103,** 1164–1166.

Kawa, K. (2003) Diagnosis and treatment of Epstein-Barr virus-associated natural killer cell lymphoproliferative disease. *International Journal of Haematology*, **78,** 24–31.

Kawa-Ha, K., Ishihara, S., Ninomiya, T., Yumura-Yagi, K., Hara, J., Murayama, F. & Hirai, K. (1989) CD3-negative lymphoproliferative disease of granular lymphocytes containing Epstein-Barr viral DNA. *Journal of Clinical Investigation*, **84,** 51–55.

Kim, H.J., Bang, S.M., Lee, J., Kwon, H.C., Suh, C., Kim, H.J., Lee, J.H., Ryoo, B.Y., Park, Y.H., Kwon, J.M., Oh, S.Y., Lee, H.R., Kim, K., Jung, C.W., Park, K. & Kim, W.S. (2006) High-dose chemotherapy with autologous stem cell transplantation in extranodal NK/T-cell lymphoma: a retrospective comparison with non-transplantation cases. *Bone Marrow Transplantation*, **37,** 819–824.

Koizumi, S., Seki, H., Tachinami, T., Taniguchi, M., Matsuda, A., Taga, K., Nakarai, T., Kato, E., Taniguchi, N. & Nakamura, H. (1986) Malignant clonal expansion of large granular lymphocytes with a Leu-11$^+$, leu-7$^-$ surface phenotype: *in vitro* responsiveness of malignant cells to recombinant human interleukin 2. *Blood*, **68,** 1065–1073.

Kwong, Y.-L. (2005) Natural killer-cell malignancies: diagnosis and treatment. *Leukaemia*, **19,** 2186–2194.

Kwong, Y.-L., Chan, A.C. & Liang, R.H. (1997) Natural killer cell lymphoma/leukaemia: pathology and treatment. *Haematology Oncology*, **15,** 71–79.

Kwong, Y.-L., Yamaguchi, M., Suzuki, R., Kim, W.S., Hasegawa, Y., Izutsu, K., Suzumiya, J., Okamura, T., Nakamura, S., Kawa, K. & Oshimi, K.. (2007) Phase I study of SMILE chemotherapy for advanced or relapsed/refractory extranodal NK/T-cell lymphoma/leukaemia. *Haematologica*, **92**(s1), 109 (Abstract 302).

Lanier, L.L., Chang, C., Spits, H. & Phillips, J.H. (1992) Expression of cytoplasmic CD3ϵ protein in activated human adult natural killer (NK) cells and CD3γ, δ, ϵ complexes in fetal NK cells. Implication for the relationship of NK and T lymphocytes. *Journal of Immunology*, **149,** 1876–1880.

Lay, J.-D., Tsao, C.-J., Chen, J.-Y., Kadin, M.E. & Su, I.-J. (1997) Upregulation of tumor necrosis factor-α gene by Epstein-Barr virus and activation of macrophages in Epstein-Barr virus-infected T cells in the pathogenesis of haemophagocytic syndrome. *Journal of Clinical Investigation*, **100,** 1969–1979.

Lee, J., Suh, C., Park, Y.H., Ko, Y.H., Bang, S.M., Lee, J.H., Lee, D.H., Huh, J., Oh, S.Y., Kwon, H.-C., Kim, H.J., Lee, S.I., Kim, J.H., Park, J., Oh, S.J., Kim, K., Jung, C., Park, K. & Kim, W.S. (2006a) Extranodal natural killer T-cell lymphoma, nasal-type: a prognostic model from a retrospective multicenter study. *Journal of Clinical Oncology*, **24,** 612–618.

Lee, K.W., Yun, T., Kim, D.W., Im, S.A., Kim, T.Y., Yoon, S.S., Heo, D.S., Bang, Y.J., Park, S., Kim, B.K. & Kim, N.K. (2006b) First-line ifosfamide, methotrexate, etoposide and prednisolone chemotherapy +/- radiotherapy is active in stage I/II extranodal NK/T-cell lymphoma. *Leukaemia and Lymphoma*, **47,** 1274–1282.

Li, Y.-X., Yao, B., Jin, J., Wang, W.-H., Liu, Y.-P., Song, Y.-W., Wang, S.-L., Liu, X.-F., Zhou, L.-Q., He, X.-H., Lu, N. & Yu, Z. (2006) Radiotherapy as primary treatment for stage IE and IIE nasal natural killer/T-cell lymphoma. *Journal of Clinical Oncology*, **24,** 181–189.

Liang, R., Chen, F., Lee, C.K., Kwong, Y.L., Chim, C.S., Yau, C.C. & Chiu, E. (1997) Autologous bone marrow transplantation for primary nasal T/NK cell lymphoma. *Bone Marrow Transplanation*, **19,** 91–93.

Lin, C.-W., Liu, T.-Y., Chen, S.-U., Wang, K.-T., Medeiros, L.J. & Hsu, S.-M. (2005) CD94 1A transcripts characterize lymphoblastic lymphoma/leukaemia of immature natural killer cell origin with distinct clinical features. *Blood*, **106,** 3567–3574.

Liu, Y.-J. (2001) Dendritic cell subsets and lineages, and their functions in innate and adaptive immunity. *Cell*, **106,** 259–262.

Liu, A., Takakuwa, T., Luo, W.J., Fujita, S. & Aozasa, K. (2006) Alterations in ATR in nasal NK/T-cell lymphoma and chronic active Epstein-Barr virus infection. *Cancer Science*, **97,** 605–610.

Loughran, Jr, T.P. & Starkebaum, G. (1987) Large granular lymphocyte leukaemia. Report of 38 cases and review of the literature. *Medicine*, **66,** 397–405.

Loughran, Jr, T.P., Hadlock, K.G., Yang, Q., Perzova, R., Zambello, R., Semenzato, G., Foung, S.K.H. & Poiesz, B.J. (1997) Seroreactivity to an envelope protein of human T-cell leukaemia/lymphoma virus in patients with CD3$^-$ (natural killer) lymphoproliferative disease of granular lymphocytes. *Blood*, **90,** 1977–1981.

Makishima, H., Ito, T., Asano, N., Nakazawa, H., Shimodaira, S., Kamijo, Y., Nakazawa, Y., Suzuki, T., Kobayashi, H. & Ishida, F. (2005) Significance of chemokine receptor expression in aggressive NK cell leukaemia. *Leukaemia*, **19,** 1169–1174.

Makishima, H., Ito, T., Momose, K., Nakazawa, H., Shimodaira, S., Kamijo, Y., Nakazawa, Y., Ichikawa, N., Ueno, M., Kobayashi, H., Kitano, K., Saito, H., Kiyosawa, K. & Ishida, F. (2007) Chemokine system and tissue infiltration in aggressive NK-cell leukaemia. *Leukaemia Research*, **31,** 1245–1253.

Marquez, C., Trigueros, C., Franco, J.M., Ramiro, A.R., Carrasco, Y.R., Lopez-Botet, M. & Toribio, M.L. (1998) Identification of a common developmental pathway for thymic natural killer cells and dendritic cells. *Blood*, **91,** 2760–2771.

Mizuno, S., Akashi, K., Ohshima, K., Iwasaki, H., Miyamoto, T., Uchida, N., Shibuya, T., Harada, M., Kikuchi, M. & Niho, Y. (1999) Interferon-γ prevents apoptosis in Epstein-Barr virus-infected natural killer cell leukaemia in an autocrine fashion. *Blood*, **93,** 3494–3504.

Mori, K.L., Egashira, M. & Oshimi, K. (2001) Differential stage of natural killer cell-lineage lymphoproliferative disorders based on phenotypic analysis. *British Journal of Haematology*, **115,** 225–228.

Murashige, N., Kami, M., Kishi, Y., Kim, S.-W., Takeuchi, M., Matsue, K., Kanda, Y., Hirokawa, M., Kawabata, Y., Matsumura, T., Kusumi, E., Hirabayashi, N., Nagafuji, K., Suzuki, R., Takeuchi, K. & Oshimi, K. (2005) Allogeneic haematopoietic stem cell transplantation as a promising treatment for natural killer-cell neoplasms. *British Journal of Haematology*, **130,** 561–567.

Nagamine, M., Takahara, M., Kishibe, K., Nagato, T., Ishii, H., Bandoh, N., Ogino, T. & Harabuchi, Y. (2007) Sequence variations of Epstein-Barr virus LMP1 gene in nasal NK/T-cell lymphoma. *Virus Genes*, **34,** 47–54.

Nagler, A., Lanier, L.L., Cwirla, S. & Phillips, J.H. (1989) Comparative studies of human FcRIII-positive and negative natural killer cells. *Journal of Immunology*, **143,** 3183–3191.

Nakashima, Y., Tagawa, H., Suzuki, R., Karnan, S., Karube, K., Ohshima, K., Muta, K., Nawata, H., Morishima, Y., Nakamura, S. & Seto, M. (2005) Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukaemia: different genomic alteration patterns of aggressive NK-cell leukaemia and extranodal NK/T-cell lymphoma, nasal type. *Genes, Chromosomes and Cancer*, **44,** 247–255.

Nash, R., McSweeney, P., Zambello, R., Semenzato, G. & Loughran, Jr, T.P. (1993) Clonal studies of CD3$^-$ lymphoproliferative disease of granular lymphocytes. *Blood*, **81,** 2363–2368.

Nava, V.E. & Jaffe, E.S. (2005) The pathology of NK-cell lymphomas and leukaemias. *Advances in Anatomic Pathology*, **12,** 27–34.

Ohshima, K., Haraoka, S., Ishihara, S., Ohgami, A., Yoshioka, S., Suzumiya, J. & Kikuchi, M. (2002) Analysis of chromosome 6q deletion in EBV-associated NK cell leukaemia/lymphoma. *Leukaemia and Lymphoma*, **43,** 293–300.

Oshimi, K. (1988) Granular lymphocyte proliferative disorders: report of 12 cases and review of the literature. *Leukaemia*, **2,** 617–627.

Oshimi, K. (1996) Lymphoproliferative disorders of natural killer cells. *International Journal of Haematology*, **63,** 279–290.

Oshimi, K. (2000) Provisional classification and diagnostic criteria of NK-cell tumours proposed by the NK-cell Tumour Study group (in Japanese). *Ketsueki-Shuyo-ka*, **40,** 87–88.

Oshimi, K. (2003) Leukaemia and lymphoma of natural killer lineage cells. *International Journal of Haematology*, **78,** 18–23.

Oshimi, K., Yamada, Y., Kaneko, T., Nishinarita, S., Iizuka, Y., Urabe, A., Inamori, K., Asano, S., Takahashi, S., Hattori, M., Naohara, T., Ohira, Y., Togawa, A., Masuda, Y., Okubo, Y., Furusawa, S., Sakamoto, S., Omine, M., Mori, M., Tatsumi, E. & Mizoguchi, H. (1993) Laboratory findings and clinical courses of 33 patients with granular lymphocyte-proliferative disorders. *Leukaemia*, **7,** 782–788.

Oshimi, K., Kawa, K., Nakamura, S., Suzuki, R., Suzumiya, J., Yamaguchi, M., Kameoka, J., Tagawa, S., Imamura, N., Ohshima, K., Kojya, S., Iwatsuki, K., Tokura, Y., Sato, E. & Sugimori, H. (2005) NK-cell neoplasms in Japan. *Hematology*, **10,** 237–245.

Plum, J., De Smedt, M., Verhasselt, B., Offner, F., Kerre, T., Vanhecke, D., Leclercq, G. & Vandekerckhove, B. (1999) *In vitro* intrathymic differentiation kinetics of human fetal liver CD34+CD38- progenitors reveals a phenotypically defined dendritic/T-NK precursor split. *Journal of Immunology*, **162,** 60–68.

Robertson, M.J. & Ritz, J. (1990) Biology and clinical relevance of human natural killer cells. *Blood*, **76,** 2421–2438.

Scott, A.A., Head, D.R., Kopecky, K.J., Appelbaum, F.R., Theil, K.S., Grever, M.R., Chen, I.-M., Whittaker, M.H., Griffith, B.B., Licht, J.D., Waxman, S., Whalen, M.M., Bankhurst, A.D., Richter, L.C., Grogan, T.M. & Willman, C.L. (1994) HLA-DR$^-$, CD33$^+$, CD56$^+$, CD16$^-$ myeloid/natural killer cell acute leukaemia: a previously unrecognized form of acute leukaemia potentially misdiagnosed as French-American-British acute myeloid leukaemia-M3. *Blood*, **84,** 244–255.

Semenzato, G., Pandolfi, F., Chisesi, T., de Rossi, G., Pizzolo, G., Zambello, R., Trentin, L., Agostini, C., Dini, E., Vespinani, M., Cafaro, A., Pasqualetti, D., Giubellino, M.C., Migone, N. & Foa, R. (1987) The lymphoproliferative disease of granular lymphocytes. A heterogeneous disorder ranging from indolent to aggressive conditions. *Cancer*, **60,** 2971–2978.

Siu, L.L.P., Wong, K.F., Chan, J.K. & Kwong, Y.L. (1999) Comparative genomic hybridization analysis of natural killer cell lymphoma/leukaemia: recognition of consistent patterns of genetic alterations. *American Journal of Pathology*, **155,** 1419–1425.

Siu, L.L.P., Chan, V., Chan, J.K.C., Wong, K.F., Liang, R. & Kwong, Y.L. (2000) Consistent patterns of allelic loss in natural killer cell lymphoma. *American Journal of Pathology*, **157,** 1803–1809.

Siu, L.L.P., Chan, J.K.C. & Kwong, Y.L. (2002) Natural killer cell malignancies: clinicopathological and molecular features. *Histology and Histopathology*, **17,** 539–554.

Soler, J., Bordes, R., Ortuno, F., Montagud, M., Martorell, J., Pons, C., Nomdedeu, J., Lopez-Lopez, J.J., Prat, J. & Rutllant, M. (1994) Aggressive natural killer cell leukaemia/lymphoma in two patients with lethal midline granuloma. *British Journal of Haematology*, **86,** 659–662.

Song, S.-Y., Kim, W.S., Ko, Y.-H., Kim, K., Lee, M.H. & Park, K. (2002) Aggressive natural killer cell leukaemia: clinical features and treatment outcome. *Haematologica*, **87,** 1343–1345.

Spits, H. & Lanier, L.L. (2007) Natural killer or dendritic: what's in a name? *Immunity*, **26,** 11–16.

Spits, H., Blom, B., Jaleco, A.-C., Weijer, K., Verschuren, M.C.M., van Dongen, J.J.M., Heemskerk, M.H.M. & Res, P.C.M. (1998) Early stages in the development of human T, natural killer and thymic dendritic cells. *Immunological Reviews*, **165,** 75–86.

Sugimoto, K.J., Kawamata, N., Sakajiri, S. & Oshimi, K. (2002) Molecular analysis of oncogenes, ras family genes (N-ras, K-ras, H-ras), myc family genes (c-myc, N-myc) and mdm2 in natural killer cell neoplasms. *Japanese Journal of Cancer Research*, **93,** 1270–1277.

Suzuki, R. (2005) Leukaemia and lymphoma of natural killer cells. *Journal of Clinical and Experimental Haematopathology*, **45,** 51–70.

Suzuki, R. & Nakamura, S. (1999) Malignancies of natural killer (NK) cell precursor: myeloid/NK cell precursor acute leukaemia and blastic NK cell lymphoma/leukaemia. *Leukaemia Research*, **23,** 615–624.

Suzuki, R., Yamamoto, K., Seto, M., Kagami, Y., Ogura, M., Yatabe, Y., Suchi, T., Kodera, Y., Morishima, Y., Takahashi, T., Saito, H., Ueda, R. & Nakamura, S. (1997) CD7$^+$ and CD56$^+$ myeloid/natural killer cell precursor acute leukaemia: a distinct haematolymphoid disease entity. *Blood*, **90,** 2417–2428.

Suzuki, R., Murata, M., Kami, M., Ohtake, S., Asou, N., Kodera, Y., Tomonaga, M., masaki, Y., Kusumoto, S., Takeuchi, J., Matsuda, S., Hirai, H., Yorimitsu, S., Hamajima, N., Seto, M., Shimoyama, M., Ohno, R., Morishima, Y. & Nakamura, S. (2003) Prognostic significance of CD7$^+$CD56$^+$ phenotype and chromosome 5 abnormalities for acute myeloid leukaemai M0. *International Journal of Haematology*, **77,** 482–489.

Suzuki, R., Suzumiya, J., Nakamura, S., Aoki, S., Notoya, A., Ozaki, S., Gondo, H., Hino, N., Mori, H., Sugimori, H., Kawa, K. & Oshimi, K. (2004) Aggressive natural killer-cell leukaemia revisited: large granular lymphocyte leukaemia of cytotoxic NK cells. *Leukaemia*, **18,** 763–770.

Suzuki, R., Nakamura, S., Suzumiya, J., Ichimura, K., Ogata, K., Kura, Y., Aikawa, K., Teshima, H., Sako, M., Kojima, H., Nishio, M., Yoshino, T., Sugimori, H., Kawa, K. & Oshimi, K. (2005) Blastic natural killer cell lymphoma/leukaemia (CD56-positive blastic tumour). Prognostication and categorization according to anatomic sites of involvement. *Cancer*, **104,** 1022–1031.

Suzuki, R., Suzumiya, J., Nakamura, S., Kagami, Y., Kameoka, J.-I., Sakai, C., Mukai, H., Takenaka, K., Yoshino, T., Tsuzuki, T., Sugimori, H., Kawa, K., Kodera, Y. & Oshimi, K. (2006) Haematopoietic stem cell transplantation for natural killer-cell lineage neoplasms. *Bone Marrow Transplantation*, **37,** 425–431.

Suzumiya, J., Takeshita, M., Kimura, N., Kikuchi, M., Uchida, T., Hisano, Y., Eura, Y., Kozuru, M., Nomura, Y., Tomita, K., Komiyama, S. & Okumura, M. (1994) Expression of adult and fetal natural killer cell markers in sinonasal lymphomas. *Blood*, **83,** 2255–2260.

Suzumiya, J., Ohshima, K., Takeshita, M., Kanda, M., Kawasaki, C., Kimura, N., Tamura, K. & Kikuchi, M. (1999) Nasal lymphomas in Japan: a high prevalence of Epstein-Barr virus type A and deletion within the latent membrane protein gene. *Leukaemia and Lymphoma*, **35,** 567–578.

Takahashi, N., Miura, I., Chubachi, A., Miura, A.B. & Nakamura, S. (2001) A clinicopathological study of 20 patients with T/natural

killer (NK)-cell lymphoma-associated haemophagocytic syndrome with specific reference to nasal and nasal-type NK/T-cell lymphoma. *International Journal of Haematology*, **74,** 303–308.

Tani, R., Ozaki, S., Kosaka, M., Fujiwara, S., Shibata, H., Wakatsuki, S. & Matsumoto, T. (1999) Fas ligand-induced apoptosis of hepatocytes in natural killer cell leukaemia. *British Journal of Haematology*, **106,** 709–712.

Tefferi, A., Li, C.Y., Witzig, T.E., Dhodapkar, M.V., Okuno, S.H. & Phyliky, R.L. (1994) Chronic natural killer cell lymphocytosis: a descriptive clinical study. *Blood*, **84,** 2721–2725.

Teruya-Feldstein, J., Jaffe, E.S., Burd, P.R., Kanegane, H., Kingma, D.W., Wilson, W.H., Longo, D.L. & Tosato, G. (1997) The role of Mig, monokine induced by interferon-γ, and IP-10, the interferon-γ-inducible protein-10, in tissue necrosis and vascular damage associated with Epstein-Barr virus-positive lymphoproliferative disease. *Blood*, **90,** 4099–4105.

Tien, H.-F., Su, I.-J., Tang, J.-L., Lee, F.-Y., Chen, Y.-C. & Chuang, S.-M. (1997) Clonal chromosomal abnormalities as direct evidence for clonality in nasal T/natural killer cell lymphomas. *British Journal of Haematology*, **97,** 621–625.

Trinchieri, G. (1989) Biology of natural killer cells. *Advances in Immunology*, **47,** 187–376.

Uno, M., Tsuchiyama, J., Moriwaki, A., Noguchi, T., Mizoguchi, K., Ogino, T., Yoshino, T., Okada, S. & Harada, M. (2001) *In vitro* induction of apoptosis for nasal angiocentric natural killer cell lymphoma-derived cell line, NK-YS, by etoposide and cyclosporine A. *British Journal of Haematology*, **113,** 1009–1014.

Wong, K.F., Chan, J.K.C. & Kwong, Y.L. (1997) Identification of del(6)(q21q25) as a recurring chromosomal abnormality in putative NK cell lymphoma/leukaemia. *British Journal of Haematology*, **98,** 922–926.

Xu, J.-X., Hoshida, Y., Yang, W.-I., Inohara, H., Kubo, T., Kim, G.-E., Yoon, J.-H., Kojya, S., Bandoh, N., Harabuchi, Y., Tsutsumi, K., Xu, I., Jia, X.-S., Kirihata, M., Tsukuma, H. & Aozasa, K. (2006) Lifestyle and environmental factors in the development of nasal NK/T-cell lymphoma: a case-control study in East Asia. *International Journal of Cancer*, **120,** 406–410.

Yamaguchi, M., Kita, K., Miwa, H., Nishii, K., Oka, K., Ohno, T., Shirakawa, S. & Fukumoto, M. (1995) Frequent expression of P-glycoprotein/MDR1 by nasal T-cell lymphoma cells. *Cancer*, **76,** 2351–2356.

Yamaguchi, M., Ogawa, S., Nomoto, Y., Oka, K., Taniguchi, M., Nakase, K., Kobayashi, T. & Shiku, H. (2001) Treatment outcome of nasal NK-cell lymphoma: a report of 12 consecutively-diagnosed cases and a review of the literature. *Journal of Clinical and Experimental Haematopathology*, **41,** 93–99.

Yamaguchi, M., Oguchi, M., Tobinai, K., Maseki, N., Suzuki, T., Masaki, Y., Nakata, M., Hata, H., Usui, N., Wasada, I., Nakamura, S., Hotta, H. & Oshimi, K.. (2005) Phase I/II study of concurrent chemoradiotherapy for newly-diagnosed, localized nasal NK/T-cell lymphoma: results of a phase I portion of JCOG0211-DI. *Blood*, **106,** Abstract 2685.

Yamamoto, T., Iwasaki, T., Watanabe, N., Oshimi, K., Naito, M., Tsuruo, T. & Kobayashi, Y. (1993) Expression of multidrug resistance P-glycoprotein on peripheral blood mononuclear cells of patients with granular lymphocyte-proliferative disorders. *Blood*, **81,** 1342–1346.

Ye, Y., Li, T., Zhang, B. & Guo, Z. (2007) Amplification and specific expression of T-bet gene in nasal NK/T-cell lymphoma. *Leukaemia and Lymphoma*, **48,** 168–173.

Yong, W., Zheng, W., Zhang, Y., Zhu, J., Wei, Y., Zhu, D. & Li, J. (2003) L-Asparaginase-based regimen in the treatment of refractory midline nasal/nasal-type T/NK-cell lymphoma. *International Journal of Haematology*, **78,** 163–167.

Zambello, R., Trentin, L., Ciccone, E., Bulian, P., Agostini, C., Moretta, A., Moretta, L. & Semenzato, G. (1993) Phenotypic diversity of natural killer (NK) populations in patients with NK-type lymphoproliferative disease of granular lymphocytes. *Blood*, **81,** 2381–2385.

Zambello, R., Loughran, Jr, T.P., Trentin, L., Pontisso, P., Battistella, L., Raimondi, R., Facco, M., Sancetta, R., Agostini, C., Pizzolo, G. & Semenzato, G. (1995) Serologic and molecular evidence for a possible pathogenetic role of viral infection in CD3-negative natural killer-type lymphoproliferative disease of granular lymphocytes. *Leukaemia*, **9,** 1207–1211.

Phase I study of dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide (SMILE) chemotherapy for advanced-stage, relapsed or refractory extranodal natural killer (NK)/T-cell lymphoma and leukemia

Motoko Yamaguchi,[1,12] Ritsuro Suzuki,[2] Yok-Lam Kwong,[3] Won Seog Kim,[4] Yuichi Hasegawa,[5] Koji Izutsu,[6] Junji Suzumiya,[7] Takayuki Okamura,[8] Shigeo Nakamura,[9] Keisei Kawa[10] and Kazuo Oshimi[11]

[1]Mie University Graduate School of Medicine, 2-174 Edobashi, Tsu, Mie 514-8507; [2]Nagoya University School of Medicine, 1-1-20 Daiko-minami, Higashi-ku, Nagoya, 461-0047, Japan; [3]University of Hong Kong, Pokfulam Road, Hong Kong, China; [4]Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong, Kangnam-gu, Seoul 135-710, Korea; [5]University of Tsukuba, 1-1-1 Tennoudai, Tsukuba, Ibaraki 305-8575; [6]Kanto Medical Center NTT EC, 5-9-22 Higashi-Gotanda, Shinagawa-ku, Tokyo 141-8625; [7]Fukuoka University Chikushi Hospital, 1-1-1 Zokumyoinn, Chikushino-shi, Fukuoka 818-8502; [8]University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215; [9]Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550; [10]Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodocho, Izumi, Osaka 594-1101; [11]Juntendo University, Tokyo, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan

(Received October 17, 2007/Revised December 15, 2007/Accepted December 21, 2007)

Extranodal natural killer (NK)/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia are rare, and their standard therapy has not been established. They are Epstein–Barr virus-associated lymphoid malignancies, and tumor cells express P-glycoprotein leading to multidrug resistance of the disease. Patients with stage IV, relapsed or refractory diseases have a dismal prognosis, with survival measured in months only. To develop an efficacious chemotherapeutic regimen, we conducted a dose-escalation feasibility study of a new chemotherapeutic regimen, SMILE, comprising the steroid dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide. The components of SMILE are multidrug resistance-unrelated agents and etoposide. Etoposide shows both *in vitro* and *in vivo* efficacy for Epstein–Barr virus-associated lymphoproliferative disorders. Eligible patients had newly diagnosed stage IV, relapsed or refractory diseases after first-line chemotherapy, were 15–69 years of age, and had satisfactory performance scores (0–2). Four dose levels of methotrexate and etoposide were originally planned to be evaluated. At level 1, six patients with extranodal NK/T-cell lymphoma, nasal type, were enrolled. Their disease status was newly diagnosed stage IV ($n = 3$), first relapse ($n = 2$), and primary refractory ($n = 1$). All of the first three patients developed dose-limiting toxicities, and one of them died of sepsis with grade 4 neutropenia. A protocol revision stipulating early granulocyte colony-stimulating factor administration was made. Two out of three additional patients developed dose-limiting toxicities that were all manageable and transient. For the six enrolled patients, the overall response rate was 67% and the complete response rate was 50%. Although its safety and efficacy require further evaluation, we recommend a SMILE chemotherapy dose level of 1 for further clinical studies. (*Cancer Sci* 2008)

Extranodal natural killer (NK)/T-cell lymphoma, nasal type (ENKL), and aggressive NK-cell leukemia (ANKL) account for 3–8% of malignant lymphomas in East Asia.[1,2] Both are Epstein–Barr virus (EBV)-associated lymphoid malignancies.[3,4] Neoplastic NK cells, similar to their normal counterparts, express high levels of P-glycoprotein, leading to the concern that multidrug resistance (MDR) might be an obstacle to successful treatment with chemotherapy.[2,5,6] More than two-thirds of patients with ENKL present with localized disease.[7–9] Recent studies suggest that first-line local radiotherapy of at least 45 Gy is effective for these patients.[10–13] Concurrent chemoradiotherapy has also been reported to be efficacious,[10,12] as supported by results of a recent prospective study.[14] In contrast, the treatment results of stage IV, relapsed or refractory ENKL, and ANKL with conventional chemotherapy are extremely poor.[4,7–9] Long-term survival after high-dose chemotherapy and hematopoietic stem-cell transplantation (HSCT) has been reported for a small number of patients with advanced-stage, relapsed or refractory disease.[15,16] Successful disease control, an important prerequisite to HSCT, is however rarely achieved in most patients with relapsed or refractory diseases. Therefore, the development of an effective chemotherapy regimen for these patients is an important initial step in improving the treatment outcome.

To address this issue, we have formulated a new chemotherapeutic regimen comprising the steroid dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide (SMILE). The design of the SMILE regimen was based on several considerations. Etoposide has demonstrated *in vitro* and *in vivo* efficacy for NK-cell neoplasms,[17,18] being effective for pediatric EBV-related hemophagocytic syndrome,[19] and pediatric EBV-associated lymphoproliferative disease.[20] L-Asparaginase induces the selective apoptosis of NK lymphoma cells *in vitro*.[21] Indeed, successful therapeutic results in NK-cell lymphoma have been reported for L-asparaginase, either alone,[22] or in combination with other chemotherapy.[23] Dexamethasone is better than prednisolone in ameliorating the adverse drug reactions of L-asparaginase.[24] Methotrexate and ifosfamide are unaffected by the MDR phenotype, and are components of regimens reported to be effective in NK/T-cell lymphomas.[10,18,25] Methotrexate was scheduled on day 1 to precede the other drugs because there is a possibility of it showing antagonistic effects on administration with etoposide and ifosfamide,[26] but synergic effects when preceding etoposide.[27] The other three drugs were scheduled for days 2–4 because the simultaneous use of etoposide and ifosfamide might lead to additive effects.[26]

Because advanced-stage ENKL and ANKL are rare and aggressive, a prospective therapeutic trial for these diseases is

[12]To whom correspondence should be addressed.
E-mail: waniwani@clin.medic.mie-u.ac.jp

doi: 10.1111/j.1349-7006.2008.00768.x

difficult to conduct. To overcome this problem, we designed a multicenter cooperative phase I study, the first of its kind, in East Asia where the incidence of ENKL and ANKL is higher than in other parts of the world. In the present report, we describe the results of a dose-escalation feasibility study of SMILE in newly diagnosed stage IV, relapsed or refractory ENKL and ANKL.

Materials and Methods

Patient selection. Patients of 15–69 years of age with ENKL or ANKL diagnosed according to the World Health Organization (WHO) classification,[28] who were newly diagnosed with Ann Arbor stage IV disease, first relapsed or recurrent disease after remission, or refractory disease after first-line chemotherapy, were eligible for the SMILE phase I study. Neither chemotherapy nor radiotherapy was given within 21 days before registration. Additional entry requirements included an Eastern Cooperative Oncology Group performance status of 0–2, at least one evaluable lesion, and laboratory parameters obtained within 7 days before registration within the following ranges: white blood cells (WBC) $\geq 3000/mm^3$, absolute neutrophil count $\geq 1200/mm^3$, platelet count $\geq 7.5 \times 10^4/mm^3$ (for patients with bone marrow involvement or hemophagocytosis, platelet count must be $\geq 5.0 \times 10^4/mm^3$), aspartate aminotransferase (AST) $\leq$ upper normal limit $\times$ 5, alanine aminotransferase (ALT) = upper normal limit $\times$ 5, total bilirubin $\leq$ 2.0 mg/dL, serum creatinine $\leq$ 1.5 mg/dL, left ventricular ejection fraction $\geq$ 50%, arterial blood gas $\geq$ 65 mmHg or O_2 saturation $\geq$ 90% (under room air). Patients with no ischemic change, atrial fibrillation, or ventricular arrhythmia requiring treatment carried out within 21 days were eligible. Patients who received corticosteroids alone were eligible for this study, but those under treatment had to discontinue it before registration. Patients who had a history of HSCT, only had cutaneous lesions, or clinical symptoms of central nervous system involvement were excluded.

Registration was conducted by facsimile from participating physicians to the regional Study Coordinators (M. Y. within and R. S. outside Japan). The protocol was approved by the Protocol Committee and the institutional review board at each participating institute. All patients gave written informed consent.

Study design. The study was designed as a phase I dose-escalation study conducted by the NK-cell Tumor Study Group in Japan, and collaborative institutes in Hong Kong, Korea, and Taiwan. The primary endpoint was the maximum tolerated dose (MTD) of SMILE, and the secondary endpoints were the overall response rate (ORR) and complete response (CR) rate. Considering that the study was the first prospective multicenter trial for ENKL and ANKL in East Asia, the study was not designed as a phase I/II study. The protocol stipulated that a subsequent phase II study to examine the efficacy of SMILE chemotherapy would be projected after the resolution of the recommended dose.

The National Cancer Institute Common Toxicity Criteria 2.0 were used for safety evaluation. A standard 3 + 3 design was used to evaluate dose-limiting toxicities (DLT). Four dose levels of methotrexate and etoposide were planned to be evaluated. DLT included grade 4 hematologic toxicities lasting 7 days or more; any non-hematological toxicity of grade 3 or more except for nausea, vomiting, stomatitis, hypofibrinogenemia, and hyperglycemia; more than 28 days delay of the second course of SMILE; and patient refusal. Treatment efficacy was evaluated according to the WHO response criteria.[29] Three to six patients were enrolled in each level. When all of the protocol treatments of the first three patients for each level were completed, registration was held, and all adverse events observed in the three patients were evaluated according to the criteria for DLT. When the initial three cases in level 1 developed DLT, the protocol committee reconsidered the continuation of this study. When one or two of the three patients in each level developed DLT, an additional three patients were enrolled at the same level. If three of the six patients developed DLT, the protocol committee reconsidered the continuation of this study. When the numbers of patients who developed DLT was two or lower in the six patients, the next cohort of patients was treated at the next level. When more than two patients in a cohort of three or six patients experienced DLT, no additional patients were enrolled and dose escalation ceased. When none of the three patients developed DLT, study registration was started with the next level. When all of the first three patients developed DLT at level $n + 1$, the MTD was determined as level n. When none of the three patients in level 4 developed DLT, the MTD was determined as level 4. When treatment-related death occurred, registration was stopped when the severe adverse event report was submitted. The protocol committee discussed the continuation of the study, and their decision was reviewed by the Data and Safety Monitoring Committee.

Treatment. The drug doses of level 1 and the administration schedule were as follows: dexamethasone, 40 mg/body intravenously on days 2–4; methotrexate, 2 g/m^2 intravenously over 6 h on day 1; ifosfamide, 1.5 g/m^2 intravenously on days 2–4; *Escherichia coli* L-asparaginase (Leunase; Kyowa Hakko Kogyo, Tokyo, Japan), 6000 U/m^2 intravenously on days 8, 10, 12, 14, 16, 18, and 20; and etoposide, 100 mg/m^2 intravenously on days 2–4. Doses of methotrexate and etoposide were scheduled to be escalated to 2 g/m^2 and 150 mg/m^2 in level 2, 3 g/m^2 and 150 mg/m^2 in level 3, and 3 g/m^2 and 200 mg/m^2 in level 4. The second course was started from day 29 of the first course. Leucovorin was begun 30 h after the initiation of methotrexate. Mesna was given at 300 mg/m^2 simultaneously with ifosfamide, and at 4 and 8 h afterwards. Granulocyte colony-stimulation factor (G-CSF) was initiated if the WBC count decreased to less than $2000/mm^3$, and was discontinued if the WBC count exceeded $5000/mm^3$. If L-asparaginase-induced grade 1–2 allergic reactions or hypersensitivity were observed, the dose of L-asparaginase was reduced by half. When L-asparaginase was discontinued due to grade 4 thrombocytopenia or grade 3–4 non-hematological toxicity in the first course, it could be resumed in the second course if the patient had recovered. Two courses of SMILE were planned.

Results

Patients. Patient registration for the SMILE phase I study was started in July 2005. A total of seven patients were registered. All patients had ENKL, so that no patient with ANKL had been registered. The first patient enrolled (patient #01) was ineligible because of thrombocytopenia. We decided to exclude this patient from further evaluation, and our decision was approved by the Data and Safety Monitoring Committee. For the six eligible patients, there were five men and one woman, at a median age of 48 years (range 28–69 years). The disease status was newly diagnosed stage IV ($n = 3$), first relapse ($n = 2$), and primary refractory ($n = 1$). Serum lactate dehydrogenase was elevated in four patients, and performance status was higher than one in one patient (Table 1). EBV was identified in tumor cells by *in situ* hybridization of all six eligible patients. CD56 was positive in five patients. The other patient (#07) was CD56-negative and cytotoxic molecule-positive, hence fitting the diagnostic criteria of ENKL according to the WHO classification.[28]

Evaluation of DLT. All of the three initial eligible patients in level 1 developed DLT. Of these, one patient (#02) experienced grade 5 infection accompanied by grade 4 neutropenia. For this patient, the initiation of G-CSF was delayed (from day 15 with a WBC count of $100/mm^3$), which was evident by case report-form monitoring and it was considered as a protocol violation.

doi: 10.1111/j.1349-7006.2008.00768.x

Table 1. Patient characteristics ($n = 6$)

Patient no.	#02	#03	#04	#05	#06	#07
Age (years)	63	39	28	57	33	69
Sex	M	M	M	M	M	F
Disease state	Refractory	First relapse	Newly diagnosed	Newly diagnosed	Newly diagnosed	First relapse
Stage at diagnosis	IVB	IEA	IVB	IVB	IVB	IEA
Sites of involvement at diagnosis	Bone marrow, spleen	Nasal cavity	Waldeyer ring, lymph nodes, nasal cavity, bone marrow	Bilateral adrenal glands, pancreas, gallbladder	Nasopharynx, lymph nodes, small bowel	Nasal cavity
Initial treatment	CHOP × 2	CHOP × 2→RT →IMVP-16	–	–	–	RT (42 Gy) →DeVIC × 4
Sites of involvement at registration	Bone marrow, spleen	Nasal cavity	Waldeyer ring, lymph nodes, nasal cavity, Bone marrow	Bilateral adrenal glands, pancreas, gallbladder	Nasopharynx, lymph nodes, small bowel	Nasal cavity, paranasal sinuses, cheek
PS	1	1	1	2	0	0
sLDH level	Elevated	Below the upper normal range	Elevated	Elevated	Below the upper normal range	Elevated

CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; DeVIC, dexamethasone, etoposide, ifosfamide, carboplatin; IMVP-16, ifosfamide, methotrexate, etoposide; PS, performance status; RT, radiotherapy; sLDH, serum lactate dehydrogenase.

Other DLT in this patient were grade 4 leukopenia and grade 4 neutropenia lasting 7 days, febrile neutropenia, grade 3 AST elevation, and grade 3 ALT elevation. Another patient (#03) developed grade 3 hyponatremia (Na 129 mEq/L) of 1-day duration alone. The DLT developing in the remaining patient (#04) were grade 4 leukopenia and grade 4 neutropenia lasting 7 days, and febrile neutropenia. We subsequently made a protocol revision stipulating mandatory initiation of G-CSF from day 6 and cessation of L-asparaginase if grade 4 thrombocytopenia or grade 3 or more non-hematological toxicity developed during its administration. These revisions were approved by the Data and Safety Monitoring Committee. After these revisions, three additional patients were registered until October 2006. Of these patients, two developed DLT. One patient (#05) developed grade 3 hyponatremia and grade 3 activated partial thromboplastin time (APTT) prolongation. Another patient (#06) experienced grade 3 hyponatremia. All DLT that developed in these two patients were manageable and transient. According to the criteria for assessment of DLT, dose escalation to level 2 was not done.

Safety. All six evaluable patients developed grade 3 or 4 leukopenia and grade 4 neutropenia (Table 2). Grade 3 non-hematological toxicities associated with protocol treatment included hyponatremia ($n = 3$), febrile neutropenia ($n = 2$), APTT prolongation ($n = 1$), hypofibrinogenemia ($n = 1$), nausea ($n = 1$), AST elevation ($n = 1$), ALT elevation ($n = 1$), and hyperglycemia ($n = 1$) (Table 2). For the last three patients who were registered after protocol revision, the hematological toxicity was less severe, and no grade 4 non-hematological toxicity was encountered. Grade 3 non-hematological toxicities consisted of two for hyponatremia and one each for APTT prolongation and hyperglycemia; all resolved rapidly and were manageable. Dose modification was needed only in L-asparaginase, and the maximum delay of the second course of SMILE was 7 days (Table 3). No severe adverse events such as allergy or thrombosis were observed.

Efficacy. The efficacy of treatment is shown in Table 3. One patient died of infection and could not be evaluated. The responses were CR in three patients, partial response in one patient, and no response in one patient, giving a CR rate of 50% and an ORR of 67%. Three patients were treated with additional SMILE chemotherapy followed by high-dose chemotherapy and autologous HSCT (Table 3). In patient #03, SMILE chemotherapy was terminated at the end of the first course because of prolonged pancytopenia and hypofibrinogenemia. He was treated with additional chemotherapy but died of disease 3 months later. Patient #07 was treated with six courses of SMILE. In this patient, allergic reaction to *E. coli* L-asparaginase developed in the fourth course of SMILE. After that, *Erwinia* L-asparaginase was used.

Table 2. Grade 3 and 4 toxicity profile ($n = 6$)

Adverse event	First three patients (#02–#04)		Additional three patients (#05–#07)	
	Grade 3	Grade 4	Grade 3	Grade 4
Leukopenia	0	3 (2)	3	0
Neutropenia	0	3 (2)	0	3 (0)
Anemia	3	0	0	0
Thrombocytopenia	1	1 (1)	0	0
RBC transfusion	3	0	0	0
PLT transfusion	2	0	1	0
Febrile neutropenia	2	0	0	0
Infection with grade 3 or 4 neutropenia	0	1†	0	0
Nausea	0	0	1	0
AST elevation	1	0	0	0
ALT elevation	1	0	0	0
Hypofibrinogenemia	1	0	0	0
Prolonged APTT	0	0	1	0
Hyperglycemia	0	0	1	0
Hyponatremia	1	0	2	0

Values indicate patient numbers with each adverse events. Values in parentheses show those with grade 4 hematological toxicities lasting 7 days or more.
†Died of sepsis (grade 5 infection).
ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; PLT, platelet; RBC, red blood cell.

Discussion

In the present study, we have attempted to tackle several obstacles in the effective treatment of advanced or relapsed NK/T-cell lymphoma and leukemia. ENKL and ANKL respond poorly to conventional chemotherapy designed for B-cell