厚生労働科学研究費補助金（がん臨床研究事業）

分担研究報告書

「NK 細胞腫瘍に対する東アジア多国間治療研究」班

分担研究課題　「NK 細胞腫瘍の治療研究と東アジア研究組織の構築（臨床試験の実施）」

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研究要旨
　　致死的疾患である NK 細胞腫瘍の新規治療法として SMILE 療法を多国間の臨床試験と
して実施する．実施上の問題点を掲げ，その解決のための方策を分担研究として検討する．

A．研究目的
　東アジアに比較的多く発症する extranodal
NK/T-cell lymphoma, nasal type (ENKL) 及び
aggressive NK-cell leukemia (ANKL) は，
Epstein-Barr virus (EBV) 感染が関連する予後不
良な致死的リンパ腫・白血病であり，標準治療法は
未だ確立していない．本研究では，日本に加え，香
港・韓国・広州・台湾の研究者と協議を行い考案し
た SMILE 療法を最低 2 コース施行し，その治療効
果を判定する臨床研究を多国間で行い，これらの難
治性疾患の標準治療法を確立することを目的とす
る．

B．研究方法
　現在本研究において，SMILE 療法の第 II 相臨床
試験を行っており，inclusion criteria を満たす患者
が当施設での試験に参加を希望し，手続きが完了す
れば，当施設での加療を行う予定である．第 I 相試
験における当施設での経験では，登録した 2 例とも
完全寛解を得，SMILE 療法の治療効果を実感でき
たため，SMILE 療法後の治療戦略についても検討
することとした．また，exclusion criteria により
治療を受けられない状態の悪い患者を，どのように
救援していくかについても検討した．SMILE 療法
後の治療戦略については造血幹細胞移植の可能性
を検討し，SMILE に参加できない患者に対しては，

当施設で考案した，より治療強度を弱めた MILD
療法の pilot study を行った．

（倫理面への配慮）
　ヘルシンキ宣言を遵守することはもちろん，臨床
研究に関する倫理指針に則り臨床試験を実施して
いる．現在行われている第 II 相臨床試験は，当施
設も含めて登録している施設の IRB の審査を受け
ており，患者に対しては，説明文書を患者に手渡し
た上，口頭で説明し，文書での同意を得た上で患者
登録を行っている．また当科で行っている pilot
study についても同様の手続きを踏んでいる。

C．研究結果
　自験例も含め，第 I 相試験で完全寛解率の向上を
期待させる SMILE 療法の効果から，寛解後療法の
重要性が討議され，再発再燃を抑えるために，同種
造血幹細胞移植を地固め療法の設定で行う有用性
が期待された．8 月 30 日に当研究班の第一回班会
議を順天堂大学で開催した際，NK 腫瘍における造
血幹細胞移植研究の可能性について報告した．また，
実際 2 名の ENKL 患者（初発 1 例，再発 1 例，い
ずれも stage IV）が候補となったが，リンパ球数の
exclusion criteria により，第 II 相試験に参加でき
なかった．そのため，その 2 例について当施設で再
発・不応性リンパ増殖性疾患に行っている MILD

療法（methotrexate, ifosfamide, L-asparaginase, dexamethasone）を行ったところ，2コースで2例とも部分寛解以上の効果を得ることができた．MILD療法はSMILE療法で用いる薬剤と同じ薬剤を用い，SMILE療法よりも1サイクルの治療強度を減じ，繰り返しの期間を短縮した治療である．ENKLやANKLの患者さんも含め，当施設におけるMILD療法17例の経験から，T/NKリンパ増殖性疾患に対して有用である可能性が期待され，12月20日の第二回班会議で その旨報告した．

D．考察

本研究が第II相試験に移った後，治療関連死亡の報告が続き，SMILE療法自体骨髄抑制の強い治療法であることから，新たにexclusion criteriaの項目にリンパ球減少が加えられた．それにより毒性は軽減されたと思われるが，当施設で2例続いたように参加できない患者が生じている．そのような患者に対しては，SMILE療法に比べて1コースの治療強度が弱いMILDを行い，短期間で繰り返すことによって，SMILE療法と同等の効果を得ることが期待できるのではないかと推察された．しかし今後performance statusの悪い，血球減少をきたしている患者群への治療経験を重ねる必要があると思われる．

SMILE療法に反応が得られた患者群に対する後療法については，やはり同種造血幹細胞移植の重要性が認識される．一般に悪性リンパ腫に対する同種造血幹細胞移植の有用性は確固たるエビデンスはないが，T/NK細胞性リンパ腫については，40%近くの治療関連死亡があるものの，再発率の低下が示されている（Le Gouill S et al, 2008）．ENKLについては， 現在まで報告されているretrospective studyの結果から，通常の前処置を用いた同種移植では毒性が強く，およそ30%以上に治療関連死亡を認めてしまうものの（Suzuki et al, 2006; Murashige et al, 2005），小児のEBV関連T/NKリンパ増殖性疾患の経験から，移植前処置の強度を減ずることによってその毒性を低下させることが可能と考えられる（Sato E et al, 2008）．実際ENKLに対するgraft versus lymphoma効果が観察されていること（Kako et al, 2007），また腫瘍細胞中のEBV関連蛋白に対する細胞性免疫によって腫瘍細胞を攻撃し得るというin vitroにおける観察

（Denmachi-Okamura et al, 2006）や細胞傷害性T細胞の浸潤が腫瘍を縮小させているという自験例での経験（Isobe et al, in press）から，免疫療法を含む同種造血幹細胞移植は，再発率を低下させる有望な治療法と考えられる．

E．結論

SMILE療法は，進行期ENKLには有望な治療法と考えられ，今後の第II相試験の結果を早期に明らかにすることが望まれる．臨床試験を進めていく上で気づいた点として，SMILE後の治療戦略，SMILE療法の臨床研究に登録不可能な患者群へのsaftey netについて今後検討していかなければならない課題であることがわかった．

F．健康危険情報
該当なし

G．研究発表
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H．知的財産権の出願・登録状況
該当なし

Ⅲ．研究成果の刊行に関する一覧

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Isobe Y, Aritaka N, Sasaki M, Oshimi K, Sugimoto K.	Spontaneous regression of natural killer-cell lymphoma.	J Clin Pathol		[in press]	2009

Ⅳ．研究成果の刊行物・別刷り

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

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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; 99: 1016–1020)

E xtranodal 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

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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 ≤ 2.0 mg/dL, serum creatinine ≤ 1.5 mg/dL, left ventricular ejection fraction $\geq 50\%$, arterial blood gas ≥ 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 pro-

tocol 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.

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

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.

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.

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

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Table 3. Treatment profile and efficacy (n = 6)

Patient no.	#02	#03	#04	#05	#06	#07
Dose reduction of SMILE (course)	L-Asp: 7→4 times (2nd)	L-Asp: reduced at a dose of 50% (2nd)	–	L-Asp: 7→4 times (1st)	–	–
Delay of the second course of SMILE	–	7 days	–	–	2 days	2 days
Planned treatment	Terminated	Completed	Terminated	Completed	Completed	Completed
Overall response	NE	CR	NR	PR	CR	CR
Additional treatment	–	SMILE × 1, SMI(L)E × 1 for PBSCH, HD-auto PBSCT	DeVIC × 1	SMILE × 1, HD-ETP for PBSCH, HD-auto PBSCT	MILD × 2, HD-ETP for PBSCH, MILD × 1, HD-auto PBSCT	SMILE × 4 (from 4th course, E. coli L-Asp was switched to Erwinia L-Asp)
Outcome	TRD, 2M	AND, 15M	DOD, 3M	AWD, 7M	AND, 7M	AND, 7M

AND, alive with no evidence of disease; AWD, alive with disease; CR, complete response; DeVIC, dexamethasone, etoposide, ifosfamide, carboplatin; DOD, died of disease; ETP, etoposide; HD, high dose; M, months after registration; MILD, methotrexate, ifosfamide, L-asparaginase, dexamethasone; NE, not evaluable; NR, no response; PBSCH, peripheral blood stem cell harvest; PBSCT, peripheral blood stem cell transplantation; PR, partial response; SMI(L)E, dexamethasone, methotrexate, ifosfamide, etoposide; SMILE, dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide; TRD, treatment-related death.

lymphomas.[30] Furthermore, the addition of anthracyclines, which are important in B-cell lymphoma regimens, has not been shown to increase therapeutic efficacy in NK-cell malignancies.[30] Finally, the role of high-dose chemotherapy with HSCT remains controversial for NK-cell lymphomas because the ideal conditioning regimen for HSCT is currently unclear.[30] Therefore, the definition of a chemotherapeutic regimen with high treatment efficacy is an important goal. The SMILE regimen reported herein is currently the only regimen formulated specifically for NK/T-cell lymphomas.

The most common adverse event during SMILE chemotherapy was grade 4 neutropenia. At level 1, one treatment-related death occurred due to a delay in G-CSF administration. However, after the protocol revision that mandated G-CSF administration from day 6, severe infection was not observed. Three out of six patients were able to undergo high-dose chemotherapy with autologous HSCT. Based on these results and the fact that only six patients were evaluated in this trial, we considered that the hematological toxicities and severe infectious complications of SMILE in level 1 with G-CSF support should be evaluated further in the setting of a prospective clinical trial.

The most frequent non-hematological adverse event was hyponatremia. Hyponatremia might be related to the syndrome of inappropriate secretion of antidiuretic hormone or renal tubular damage, which was likely caused by chemotherapeutic agents in the SMILE protocol, particularly ifosfamide. Interestingly, hyponatremia was also observed in a recent clinical trial for localized ENKL.[14] Therefore, other disease-specific causes of hyponatremia would have to be examined in future studies.

The antitumor effect of two courses of SMILE, which resulted in a CR rate of 50% and ORR of 67%, was remarkable. To date, two promising results of nonanthracycline-containing regimens in nasal ENKL have been reported. In a Mexican prospective study of newly diagnosed advanced-stage ENKL with nasal involvement,[25] patients were given six courses of cyclophosphamide, methotrexate, etoposide, and dexamethasone chemotherapy, with sandwiched radiotherapy of 55 Gy after three courses in cases with facial involvement. Another Chinese study of nasal ENKL relapsing or refractory to anthracycline-containing chemotherapy used a salvage regimen containing L-asparaginase, vincristine, and dexamethasone followed by involved-field radiotherapy (median: 56 Gy).[23] Although the CR rates in these studies exceeded 55%, high-dose radiotherapy was used, making evaluation of the contribution of the efficacy of chemotherapeutic regimens difficult. Moreover, high-dose radiotherapy may not be an option for patients with advanced disseminated diseases, and for relapsed patients who have already received involved-field radiotherapy during primary treatment for nasal ENKL. The therapeutic efficacy of the SMILE protocol requires further evaluation in a larger number of patients with advanced ENKL.

We did not use the International Working Group (IWC) criteria to assess the response in this trial for the following reasons:[31] (1) ENKL and ANKL usually show extranodal involvement that is often difficult to measure bidimensionally; and (2) given that the SMILE phase I study is the first prospective, international, multicenter-based clinical trial for ENKL and ANKL in East Asia, simple and familiar response criteria were thought to be more appropriate. Recently, the IWC criteria have been revised, and 18-fluoro-deoxyglucose (FDG) positron emission tomography scanning was incorporated into the evaluation of lymphomatous involvement.[32] Moreover, a recent report suggested that ENKL is an FDG-avid lymphoma.[33] In future clinical trials on ENKL, the appropriateness of the revised IWC criteria for ENKL needs to be examined.

According to the criteria for DLT assessment stipulated in the protocol, we did not escalate the dose to level 2 and could not determine the MTD in this trial. However, all grade 3 non-hematological toxicities developing in patients who were enrolled after the protocol revision in level 1 were manageable and transient. In addition, no grade 4 hematological toxicities lasting 7 days or more were observed in patients who were enrolled after the protocol revision (Table 2). SMILE at dose level 1 was thought to be promising because three out of six patients in level 1 achieved a CR. From these results, we believe that dose level 1 of SMILE chemotherapy is appropriate for further clinical studies. Our findings are now further evaluated in a prospective phase II study of level 1 SMILE with G-CSF support, which has started since July 2007.

Acknowledgments

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Review Article

Extranodal NK/T-cell lymphoma: diagnosis and treatment cues

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Abstract

Extranodal NK/T-cell lymphoma, nasal type (ENKL) is mostly endemic to East Asia. It predominantly occurs in the nasal or paranasal areas and less frequently in the skin. Most of the tumours show NK-cell, but rarely T-cell, phenotypes. The Epstein–Barr virus (EBV) genome can be usually detected in lymphoma cells. Geographic localization of ENKL matches the endemic distribution of EBV, suggesting that EBV plays an important role in lymphomagenesis. Originally, NK-cell and T-cell types were believed to present the same clinicopathologic characteristics, but recent data suggest more aggressive characteristics for the NK-cell phenotype. Although ENKL is sensitive to radiotherapy, it shows a poorer response to chemotherapeutic agents than other lymphomas due to expression of p-glycoprotein. Therefore, new therapeutic approaches must be considered. Several new clinical trials are now being conducted in East Asia. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: natural killer cell; azurophilic granule; Epstein–Barr virus; CD56; cytotoxic molecule

Introduction

Extranodal NK/T-cell lymphoma (ENKL), nasal type most frequently affects the nose and paranasal area [1–3]. The immunophenotype of the lymphoma cells mostly reflects that of NK-cells, but sometimes is also characteristic of T-cells. In some cases differential diagnosis is difficult when only using paraffin embedded specimens. Therefore at present, the diagnostic term 'NK/T-cell lymphoma' is used. It should however be noted that no 'NK/T-cell' actually exists. This type of lymphoma shows a marked geographic preference for East Asia and Latin America. The incidence is also different within the endemic areas; in Asia, the rates of occurrence are: 3.3% in Japan [4], 5% in Taiwan [5], 6% in Hong Kong [6] and 8% in Korea [7]. In this review, we summarize the disease characteristics of ENKL of nasal type with special emphasis on diagnostic pitfalls.
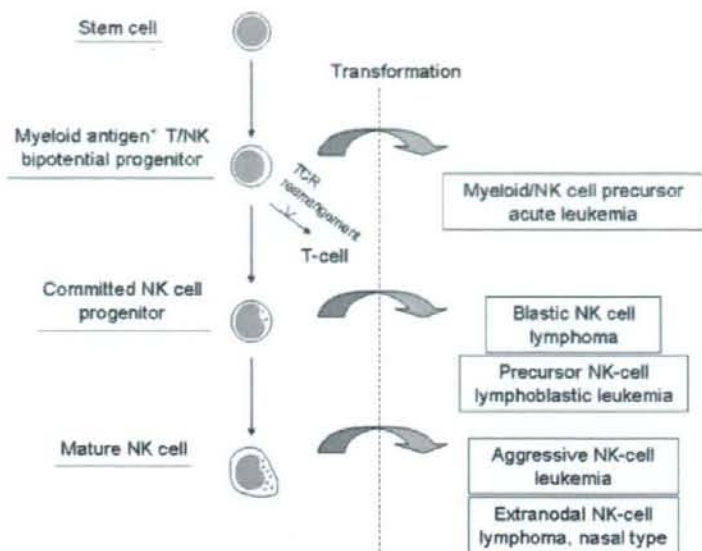
Ontogeny of NK-cells

NK-cells were first defined as a functional subset of lymphocytes that mediate major histocompatibility complex-nonrestricted cytotoxicity [8]. They were later recognized to have large granular lymphocyte (LGL) morphology, germline configurations of T-cell receptor (TCR) and immunoglobulin genes and a surface CD3

(sCD3)-negative and CD56-positive phenotype [9]. From these findings, NK-cells are now regarded as a third lineage of lymphocytes that is distinct from T- and B-cells. Because NK-cells develop from T/NK bi-potential common progenitors (Figure 1) [10,11], they share many similarities with T-cells, particularly with cytotoxic T-cells. Therefore, the phenotypes of NK-cell and T-cell lymphoma/leukaemia also have much in common, which makes it difficult to perform differential diagnosis [12–14].

Pathological transformation of NK-cells

Myeloid antigen-positive T/NK bi-potential progenitors are believed to develop by transformation into myeloid/NK cell precursor acute leukaemia [15,16]. NK-cell lineage committed progenitors are also hypothesized to transform to blastic NK-cell lymphoma (BNKL) or precursor NK-cell acute lymphoblastic leukaemia/lymphoma (NK-ALL) [17,18]. Previously, CD4-positive and CD4-negative types of BNKL/NK-ALL were identified [18]. Although there remain several controversies regarding CD56 expression and dendritic cell lineage, the CD4+ CD56+ type of this tumour is somehow related to plasmacytoid dendritic cells or to the monocytic lineage [19,20]. The CD4-negative type probably represents the true BNKL/NK-ALL. Two mature NK-cell neoplasms, ENKL [1] and aggressive NK-cell leukaemia [18d], are transformed from functionally

Figure 1. Ontogeny of NK-cells and transformation to NK-cell malignancies. NK-cells are differentiated from stem cells through myeloid-antigen positive NK/T bi-potential progenitors and lineage-committed progenitors. Myeloid/NK cell precursor acute leukaemia is transformed from the myeloid antigen-positive progenitor. Blastic NK-cell lymphoma and precursor NK-cell lymphoblastic leukaemia are derived from a relatively mature, NK-cell lineage committed progenitor. Two mature NK-cell neoplasms, aggressive NK-cell leukaemia and extranodal NK-cell lymphoma, nasal type, are transformed from mature NK-cells

mature NK-cells. Aggressive NK-cell leukaemia is a distinct leukaemic form of mature NK-cell malignancy with frequent hepatosplenic involvement [21,22]. Although these two diseases share many features, several clinico-pathologic and phenotypic differences have been reported [23]. Therefore, aggressive NK-cell leukaemia remains as a distinct disease entity in the forthcoming World Health Organization classifications. A summary of the clinico-pathologic characteristics of NK-cell lineage neoplasms is given in Table 1.

Clinical characteristics of extranodal NK-cell lymphoma, nasal type

The nose and paranasal area including the upper aero-digestive tract contains the origin of more than 80% of extranodal NK-cell lymphomas, nasal type. Macroscopic findings by nasal endoscope are shown in Figure 2. Initial complaints of ENKL, nasal type include local symptoms such as nasal obstruction, discharge and bleeding. There-after, as the disease extends, necrosis, swelling or bony destruction of the nasal area develops. However, such extreme local progressions are currently rare because of early disease recognition and reference to specialized physicians. The skin is the second most frequent organ of origin, accounting for approximately 10% of cases [19]. Cases originating from the liver and/or spleen account for 5% of ENKLs, nasal type. More rare organs of onset include the lung, gastrointestinal tract, kidney, pancreas, testis and brain. Nasal lymphomas more frequently present as a localized disease (ratio 4:1), whereas lymphomas at

other sites are more frequently detected at an advanced stage (ratio 2:3) [24–35]. Because this lymphoma essentially presents an extranodal origin, clinical stage III is rare and most of the advanced stage cases are in stage IV. Some cases show long-term limitation to the original site. However, once the tumour develops outside the original site, the disease rapidly progresses and disseminates. Fever, haemophagocytosis and disseminated intravascular coagulation are not rare in this situation. Several cases of stage IV or aggressive NK-cell leukaemia could not be treated because of the progression of the disease and poor status of the patient [36].

Diagnosis of extranodal NK-cell lymphoma, nasal type

Diagnosis of ENKL, nasal-type is based on histopathologic examination of biopsy specimens, but is sometimes difficult because of the existence of wide necrosis around the tumour (Figure 3A) that is characterized by expression of Fas and Fas ligand on the tumour cells [37]. Selection of appropriate sites for biopsy is important for prompt diagnosis, as are repeated approaches in case the speci-mens only include necrotic tissue.

Histologically, tumour cells from ENKL generally show angiocentric growth pattern (Figure 3A) [1,38]. The growth pattern is such a notable feature of this lymphoma that the diagnostic term used to be 'angiocentric lymphoma' [39]. In clinical practice, sampling error can prevent recognition of angiocentricity; therefore this finding is currently not mandatory for diagnosis [38]. The presence of cucumber-

Table 1. Clinicopathologic characteristics of NK-cell lineage neoplasms

	Myeloid/NK cell precursor acute leukaemia	Blastic NK-cell lymphoma/Precursor NK-cell lymphoblastic leukaemia	Aggressive NK-cell leukaemia	Extranodal NK cell lymphoma, nasal type	
				Limited stage	Advanced stage
Morphology	Blastic	Blastic	LGL	LGL	LGL
Azurophilic granule	−	−	+	+	+
Lymph node involvement	+	+	+	−	+/−
Extranodal involvement	Bone marrow, blood, mediastinum	Skin, bone marrow	Bone marrow, blood, liver, spleen	Nose, skin	Nose, skin, bone marrow, blood
B-symptom	Rare	Rare	Frequent	Rare	Frequent
Surface marker	CD7+, CD33+, CD34+, CD56+	CD4+/−, CD7+, CD56+, TdT+	CD2+, CD16+, CD56+	CD2+, cyCD3+, CD56+	
EBV	−	−	+/−	+	+
Clinical course	Aggressive	Aggressive	Aggressive	Sometimes indolent	Aggressive
Therapy	AML chemotherapy	Chemotherapy for lymphoid neoplasms	No standard therapy	Radiotherapy followed by chemotherapy	No standard therapy
Prognosis	Relapse is frequent, and the prognosis is poor.	Relapse is frequent, and the prognosis is poor.	Very poor	Fair	Very poor

LGL, large granular lymphocyte; EBV, Epstein–Barr virus; AML, acute myeloid leukaemia.
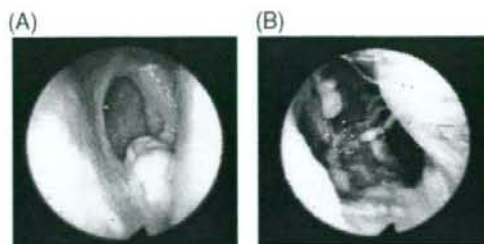

Figure 2. Naso-endoscopic findings of nasal NK/T-cell lymphoma. (A) Naso-endoscopic findings of a patient who presented with nasal discharge. Tumour formation and tissue swelling by necrosis can be observed. (B) Surface ulcerations can be seen, and a part of the nasal cartilage was destroyed

like cells with elongated nuclei is particular to ENKL, and is helpful for diagnosis (Figure 3B). If the biopsy specimen is small, a touch imprint smear with Giemsa staining is sometimes useful for diagnosis because of the presence of azurophilic granules in the tumour cells (Figure 4). The accumulation of NK-cells or cytotoxic T-cells does not directly indicate a malignant condition; however, since it is not usually recognized in the nasal mucosa, the assemblage of NK-cells (or rarely cytotoxic T-cells) is still important evidence. Epstein–Barr virus (EBV) is harboured in ENKLs of nasal type, and detection by *in situ* hybridization can be achieved for paraffin-embedded tissues or touch imprint smears (Figure 5). EBV is rarely observed in
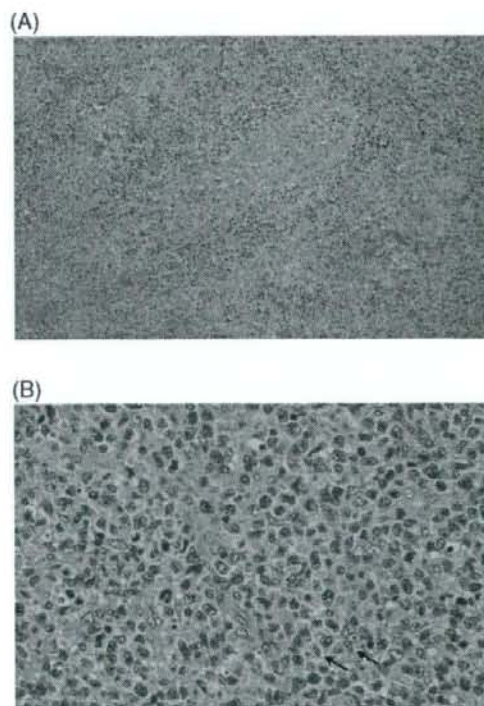

Figure 3. Biopsy specimen of nasal NK/T-cell lymphoma. (A) In a vast necrotic region, there exist several atypical medium-to-large lymphocytes. Tumour cells show an angiocentric growth pattern. (B) The nuclei of several tumour cells are elongated, and present a cucumber-like morphology (Arrow)

Figure 4. Touch imprint smear of ENKL. There are many atypical NK-cells with prominent azurophilic granules

lymphocytes residing in normal or inflammatory nasal mucosa or adjacent tissue; therefore detection is particularly important for specimens that mostly consist of necrotic tissue.

For ENKLs, histopathologic diagnosis of bone marrow involvement is occasionally difficult. Detection of EBV is also helpful in this situation [40]. Recently, the prognostic significance of such occult or minute involvement has been shown for early stage patients [41]. Routine examination of bone marrow involvement by using EBV *in situ* hybridization is now recommended.

Immunophenotype of extranodal NK-cell lymphoma, nasal type

Phenotypic markers expressed in ENKL include CD2, cytoplasmic CD3 (cyCD3), CD7 and CD56, which also represent the phenotype of normal NK-cells [25,42,43]. Cytotoxic molecules such as TIA-1, granzyme B and perforin, are also positive in ENKL [37,44]. Table 1 shows the differential diagnosis of mature NK-cell tumours. If lymphoma cells are negative for these cytotoxic molecules and show a T-cell phenotype, diagnosis of another type of T-cell lymphoma should be considered. For differentiation of NK-cell from T-cell lymphoma, expression of sCD3, CD5 or TCRs on the lymphoma cells can be evaluated [31,43,45], in addition to the rearrangement of TCR genes. However, routine diagnostic use of these procedures is sometimes difficult and unavailable. Previously, the
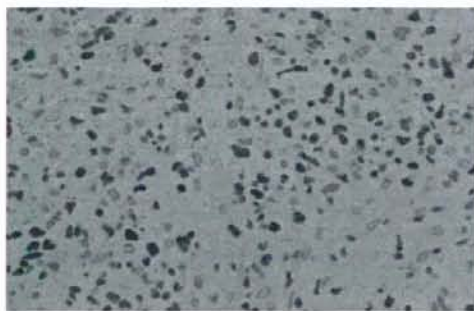
Figure 5. Epstein–Barr virus small RNA (EBER) in-situ hybridization (ISH). Because lymphoma cells harbour the EBV, they are positive for EBER-ISH

clinical features and prognosis of true T-cell nasal lymphoma were regarded to be similar to that of NK-cell type, resulting in the adoption of the term 'NK/T-cell lymphoma'. It should be noted, however, that this nomenclature falsely suggests existence of 'NK/T-cells'. Another point to be noted is that the use of the term 'NK/T' is restricted to lymphomas occurring in the nasal/paranasal area, and is not applied to lymphomas originated from extra-nasal sites. Differential diagnosis from other types of T-cell lymphomas is therefore required for the extra-nasal type of extranodal NK-cell lymphoma.

Recently, studies with large numbers of patients showed that the prognosis of nasal NK-cell lymphoma is significantly poorer than that of nasal 'T-cell' lymphoma [29,46]. Differential diagnosis of NK-cell lymphoma from genuine T-cell lymphoma may therefore be required in the future. A search for a diagnostic marker is therefore warranted.

Diagnostic pitfalls for extranodal NK/T-cell lymphoma

Because CD56 is also expressed in a part of acute myeloid leukaemia (AML) [47], differential diagnosis of NK-cell malignancies from CD56-positive AML is occasionally difficult, particularly for those with extramedullary or cutaneous involvement. $CD4^+$ $CD56^+$ haematodermic neoplasm also frequently shows cutaneous/subcutaneous involvement [18,19]. In this context, CD56-positive AML and $CD4^+$ $CD56^+$ haematodermic neoplasm are unexceptionally negative for EBV, which is useful for the differential diagnosis. Positive EBV status is thus required for the diagnosis of NK/T-cell lymphomas.

Treatment of extranodal NK/T-cell lymphoma

Limited stages

For limited stages of usual aggressive non-Hodgkin lymphoma, three to four courses of a chemotherapy regimen that includes anthracycline, such as CHOP, supplemented with involved field irradiation is regarded as the standard therapy [48]. However, for nasal NK/T-cell lymphoma, the overall 5-year survival rate using this strategy is less than 50% [49,50]. Reasons include the expression of the multidrug-resistant p-glycoprotein in NK/T-cell lymphoma cells [51,52]. P-glycoprotein actively exports doxorubicin and vincristine, which are the main components of CHOP chemotherapy. Radiotherapy remains effective but cannot prevent recurrence of the disease outside the radiation field. The overall 5-year survival rate therefore remains limited to 40–50% when using radiotherapy alone [31,53,54].

Ribrag *et al.* treated eight patients in the limited stage of nasal NK/T-cell lymphoma with radiotherapy followed by chemotherapy and reported an excellent result (10 years overall survival: 100%) [55]. They have concluded that a sufficient dose of radiotherapy immediately after diagnosis

is desirable for treatment of this disease. At present, radiotherapy followed by chemotherapy is regarded as a standard strategy for limited stage ENKL [31,56].

Yamaguchi et al. also reported excellent control of the disease by simultaneous chemoradiotherapy using radiation therapy and DeVIC chemotherapy (RT-DeVIC) [50]. Based on this finding, the Japanese Clinical Oncology Group has conducted a phase I/II study of RT-DeVIC chemoradiotherapy. The study is now closed with sufficient numbers of patients registered. Its results are anticipated.

Advanced stages

The prognosis of advanced stage ENKL, nasal type, as well as that of aggressive NK-cell leukaemia, is extremely poor when using any chemotherapeutic regimen [22]. Aviles et al. from Mexico reported the utility of sandwich chemoradiotherapy, which consisted of three courses of cyclophosphamide, methotrexate, etoposide and dexamethasone (CMED), radiotherapy and additional three courses of CMED. The 5-year overall survival rate using this method was reported as 65% [57]. This was an excellent result, but the reported toxicities were surprisingly low despite the relatively high dose of chemotherapeutic drugs used. Therefore, confirmation through replication is required.

Recently, several reports from East Asia suggest the efficacy of L-asparaginase for treatment of mature NK/T-cell lymphoma [58–60]. L-asparaginase is an enzyme that digests serum L-asparagine and acts as an anti-tumour agent through asparagine starvation of tumours with low expression levels of asparagine synthetase [61,62]. Because L-asparaginase specifically acts on lymphoid cells, myelosuppression by L-asparaginase is minimal. L-asparaginase has long been regarded as a key drug for paediatric acute lymphoblastic leukaemia. A Chinese group treated nasal NK/T-cell lymphoma patients refractory to CHOP-like chemotherapy with a chemotherapy regimen that consisted of L-asparaginase, vincristine and dexamethasone supplemented by local radiotherapy. They reported good results with a 5-year overall survival rate of 55.6% [63]. Likewise, L-asparaginase is effective for this type of lymphoma but has many adverse reactions such as haemostatic complications, allergy and pancreatitis. These findings suggest a need for the establishment of safe and effective chemotherapeutic regimens. The NK-cell Tumour Study Group is now conducting clinical studies of a novel L-asparaginase-containing chemotherapy for initial stage IV, relapsed or refractory NK/T-cell leukaemia/lymphoma [36]. This regimen is termed SMILE, and consists of methotrexate, ifosfamide, etoposide, steroid and L-asparaginase. A phase I dose finding study has been completed [64] and we are now designing a subsequent phase II study.

Haematopoietic stem cell transplantation (HSCT)

Because the prognosis of ENKL is poor, there exist several reports of upfront autologous HSCT. In large-scale reports from Japan and Korea, long-term survival ranges from 50 to 70% [65–67]. However, retrospective analysis might be biased by patient selection. Prospective clinical trials are thus warranted before concluding that autologous HSCT is effective for ENKL.

On the other hand, allogeneic HSCT can also be applied for the treatment and is the only curative strategy for advanced stage or nonremission patients. Two large-scale analyses from Japan included high-risk patients and reported a long-term survival rate ranging from 30 to 40% [66,68]. The second study included patients who received reduced intensity stem cell transplants (RIST), and both reports indicated the absence of late recurrence at 2 years post-transplantation. These findings suggest the curative potential of allogeneic HSCT, but patient selection bias is also possible. Since many types of stem cell sources are now utilized for HSCT including cord blood and mismatched donors, further accumulation of data and prospective evaluations are also required.

Clinical significance of the Epstein–Barr virus

It is well-known that patient sera from EBV-positive malignancies contain fragmented viral DNA [69,70]. Measurement of the circulating viral DNA load in peripheral blood is useful for diagnosis, monitoring and prognostication of the disease. However, detection is sometimes misunderstood as the presence of viral particle itself; rather, the detected DNA is derived from dead tumour cells. For these reasons, most detected fragments are less than 500 bp in length, and longer fragments or the entire EBV genome are never detected [71]. EBV-DNA can therefore be used as a marker to predict the tumour burden [72,73], but prediction can potentially be affected by the presence of EBV unrelated to the lymphoma. There are several choices of source tissue for analysis including plasma, total blood and mononuclear cells, and each choice represents a different outcome [74]. The significance of the viral load in peripheral blood and the choice of source tissue used for analysis should be examined prospectively.

Conclusion

Several new insights have been recently developed for extranasal NK/T-cell lymphoma, nasal type. Diagnosis is thus becoming easier. However, the prognosis is particularly poor in both the limited and advanced stages. Appropriate therapeutic strategies should be explored by prospective studies.

Acknowledgements

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