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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

予後不良の進行性節外性 NK/T 細胞リンパ腫・鼻型（ENKL），劇症型 NK 細胞白血病（ANKL)に対する有効な寛解導入療法を開発するため，「未治療 IV 期，再発・難治 NK/T 細胞リンパ腫/白血病に対する SMILE 療法の第 II 相試験（SMILE-PII)」が計画され，現在までに有効性と安全性が確認されつつある．当施設で SMILE 療法を行った症例について key drug である L-asparaginase の使用に関する問題点を検討した．また eligibility を満たさない患者群を救済する方策として，SMILE 療法の dose-intensity を弱めた MILD 療法を施行した．

A.　研究目的

成熟 NK 細胞腫瘍には，節外性 NK/T 細胞リンパ腫・鼻型（ENKL)，劇症型 NK 細胞白血病（ANKL）と緩徐な経過をたどる顆粒リンパ球増多症（GLPD）がある．成熟 NK 細胞腫瘍は，本邦においても悪性リンパ腫の数%程度の頻度を占める稀な疾患であるが，ENKL と ANKL は Epstein-Barr virus（EBV）感染と強く関連し，比較的東アジア地域に多く発症する予後不良の致死的疾患である．限局期 ENKL では診断後早期の病変部に対する放射線照射が有効であるが，進行期例あるいは ANKL に対する有効な治療法は今のところ存在しない．成熟 NK 細胞腫瘍の発症頻度は欧米では 1%未満であり，わが国をはじめ，東アジアで有効な治療法の開発を行うことが期待されている．

そこで本研究の主体である NK 腫瘍研究会は，進行期 ENKL および ANKL に対し，SMILE 療法を考案し，アジア多国間第 I 相試験を実施し，完全奏効を含む奏効を得，毒性も許容範囲内であることを確認した．そこで SMILE 療法 2 コースの寛解導入療法としての有効性と安全性を評価する本研究が開始された．しかし当施設において第 I 相試験に参加し後療法として SMILE を継続した 1 例で，L-asparaginase に対するアレルギー反応によって薬剤の変更を余儀なくされたこと，また，II 相試験で登録期間や eligibility を満たさない患者が進行期 ENKL4 例中 3 例と多く，試験への参加ができなかったことを経験し，将来的に SMILE 療法を広く一般診療に広めていく際の問題点に気づくに至った．当分担研究者はこれらの問題点について検討した．

B.　研究方法

本研究の SMILE 療法の選択基準を満たす患者群で，後療法として SMILE あるいは MILD 療法を行った症例で，L-asparaginase に対するアレルギー反応等の安全性評価のため血清中 L-asparaginase 活性および抗体を測定した．一方 SMILE の候補症例で，除外基準のため臨床試験に参加できなかった患者群に対し，SMILE 療法の dose-intensity を弱めた MILD 療法を施行した．

[血清 L-asparaginase 抗体，活性測定]

L-asparaginase は大腸菌由来の酵素製剤のため，人体に全身投与された際に薬剤に対する抗体が産生され，アレルギー反応が惹起されることが分かっている．当施設（順天堂大学医学部血液内科）において，L-asparaginase 投与中の患者の安全性確保のため，投与前，アレルギー反応発生時，発生しない場合は各治療コース 2 回目の L-asparaginase 投

与翌日の血清中 L-asparaginase 活性，IgE 抗体，IgG 抗体を測定した．対象は，急性リンパ性白血病，悪性リンパ腫の患者で L-asparaginase を含む化学療法で治療を受ける症例である．測定は薬剤の開発・発売元の協和発酵キリンの研究本部薬物動態研究所に委託した．

[MILD 療法]

(1) MILD 療法の適格例は以下の通り．

①2001 年版 WHO 分類で aggressive lymphoma/leukemia の病型

②年齢 20ー75 歳

③Performance status (ECOG)　0-2

④初回治療不応例，再発例または標準治療が開発されていない致死的疾患

⑤評価可能病変を有する

⑥十分な心・肺・肝・腎機能機能を有する

⑦患者本人よりの書面同意が得られている

(2) 治療内容

SMILE 療法の etoposide を抜いた薬剤を使用する治療レジメンで，60 歳未満と 60 歳以上で methotrexate，ifosfamide の投与量を変更している．Ifosfamide (1000 or 800 mg/m2) と dexamethasone (20 mg) を day 1~3，MTX (2 or 1.5 g/m2) を day4，L-asparaginase (6000 KU/m2) を day5, 7, 9 で投与し，2~3 週おきに繰り返す治療である．

(倫理面への配慮)

本研究に携わる医師はヘルシンキ宣言を遵守し，わが国での臨床研究に関する倫理指針あるいは海外各国ではそれに相当する指針に則って本研究を実施している．L-asparaginase の安全性については薬事法第 77 条の 3 に則り，患者自身から同意を得て活性値，抗体測定を行った．MILD 療法については，平成 18 年施設の IRB の承認を受け，当施設研究として難治性リンパ系腫瘍の救援療法として行っている中に組み込む形で実施している．

C.　研究結果

(1) L-asparaginase 投与について

当施設の SMILE 第 I 相部分での参加症例は 2 例で，1 例は SMILE2 コース後，L-asparaginase に対する抗体が，IgG 抗体 29 U/mL，IgE 抗体 61 倍と検出されていたが，後療法として MILD 療法を 3 コースおこなったもののアレルギー反応を認めなかった．2 例目は，SMILE 療法 2 コース後に完全寛解となり，後療法として SMILE 療法を行っていたが，4 コース目の L-asparaginase 投与中にアナフィラキシーをきたした．その際，投与前に IgG 抗体 4 U/mL，IgE 抗体 71 倍とすでに抗体を認めていたものの，アナフィラキシーを起こした際には IgG 抗体 12 U/mL，IgE 抗体 42 倍と IgG 抗体価の上昇を認めた．年齢やリンパ球数低値により SMILE 療法を施行できなかった 3 例の ENKL については，2 例において抗体検査の経過を追ったが，1 例は MILD5 コース目に蕁麻疹の副作用をきたし，IgG 抗体が 12 U/mL から 27 U/mL に上昇し，血清中の asparaginase 活性も上昇しなかった．またもう 1 例では，MILD2 コース開始時点で，IgG 抗体 24 U/mL，IgE 抗体 229 倍と高値を示しており，grade 3 の蕁麻疹を認めた．3 コース目の投与の際には IgG 抗体が感度以下に下がったものの asparaginase 活性の上昇は認められなかった．

(2) MILD 療法について

本研究の症例登録中当施設で計 4 例の ENKL 症例を経験し，そのうち年齢，リンパ球数の基準を満たさず 3 例の登録ができなかった．その 3 例に MILD 療法を行った．2 コース終了できた 2 例は完全寛解と部分寛解で両者とも奏効した．また，再発・難治性リンパ腫／白血病に他施設も含め計 18 例について治療を行い，2 コース後の安全性と奏功率について検討したところ，毒性は許容範囲内であり，8 例の奏効 (57%) 中 7 例が慢性活動性 EBV 感染症関連のリンパ増殖性疾患や末梢 T 細胞リンパ腫を含む T/NK 細胞腫瘍だった．

D.　考察

(1) L-asparaginase 投与について

本研究は，順調に症例登録が進み，現在 SMILE 療法の ENKL に対する有効性が明らかになりつつある．しかし，SMILE2 コース終了後どのように治療を進めていくべきなのか，不明な点が多いことも事実である．NK 細胞腫瘍は，比較的 L-asparaginase の効果が強く期待される疾患であり，本剤が key drug であると考えられるが，当施設での経験では早期に L-asparaginase の抗体価が上昇すると，アレルギー反応のみならず，薬剤の効果が期待できない状態になる可能性を示している．

SMILE 療法を 2 コース以上継続していくと，この問題と対面せざるを得ない状況が待ち受けていると予想される．小児の急性リンパ性白血病の治療で示されている，筋注やステロイド併用による L-asparaginase 投与方法の工夫によって，より抗体産生を抑える対策も考える必要があると思われた．その基礎的データを集積するためには，今後の SMILE 治療研究において，L-asparaginase 抗体と活性値の測定は今後必須であると思われる．次の SMILE 治療研究に組み込むよう検討する．

(2) MILD 療法について

MILD 療法については，SMILE 療法に登録が難しい症例の救済策として 1 つの選択肢となることが示唆された．ただし，L-asparaginase 投与の問題は SMILE 療法と同様であり，今後解決していく必要がある．MILD 療法では，L-asparaginase 投与後にアレルギー反応が出た場合，重篤なものでなければステロイド投与と 5%ブドウ糖液で希釈して筋注する方法で継続していく予定である．長期観察による治療効果については不明であるが，T/NK 細胞リンパ腫に対する奏効率をみると，本研究組織において末梢性 T 細胞リンパ腫の再発・難治例を対象とした SMILE 療法の第 II 相試験が計画され，現在症例登録が開始されており，その効果も十分に期待できるものと確信する．

E. 結論

予後不良の疾患である進行期 ENKL に対する有効な治療法は現在まで存在しなかった．初発 IV 期・再発・難治 ENKL に対する前向き臨床試験は，第 I 相試験に続く本研究のみであり，この結果が世界的標準となると予想される．本研究によって示された SMILE 療法の奏効率は，よい治療法が存在しなかった患者にとって福音となるのは間違いない．登録基準を満たさない症例の救済や key drug に対する抗体産生の問題など，今後治癒に向けたきめの細かい対応を継続的に行っていかなければならない．また，研究を進めるにあたり，東アジアでの人的交流が進み，多国間治療研究がますます活発化していくと予想される．

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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Ⅳ. 研究成果の刊行物・別刷

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Prognostic factors for mature natural killer (NK) cell neoplasms: aggressive NK cell leukemia and extranodal NK cell lymphoma, nasal type

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Background: Patients with natural killer (NK) cell neoplasms, aggressive NK cell leukemia (ANKL) and extranodal NK cell lymphoma, nasal type (ENKL), have poor outcome. Both diseases show a spectrum and the boundary of them remains unclear. The purpose of this study is to draw a prognostic model of total NK cell neoplasms.

Patients and methods: We retrospectively analyzed 172 patients (22 with ANKL and 150 with ENKL). The ENKLs consisted of 123 nasal and 27 extranasal (16 cutaneous, 9 hepatosplenic, 1 intestinal and 1 nodal) lymphomas.

Results: Complete remission rate for ENKL was 73% in stage I, but 15% in stage IV, which was consistent with that for ANKL (18%). The prognosis of ENKL was better than that of ANKL (median survival 10 versus 1.9 months, $P < 0.0001$) but was comparable when restricted to stage IV cases (4.0 months, $P = 0.16$). Multivariate analysis showed that four factors (non-nasal type, stage, performance status and numbers of extranodal involvement) were significant prognostic factors. Using these four variables, an NK prognostic index was successfully constructed. Four-year overall survival of patients with zero, one, two and three or four adverse factors were 55%, 33%, 15% and 6%, respectively.

Conclusion: The current prognostic model successfully stratified patients with NK cell neoplasms with different outcomes.

Key words: CD16, leukemia, lymphoma, natural killer cell, prognosis

original article

introduction

Neoplasms arising from natural killer (NK) cells have become widely recognized in the past decade [1–3]. There are two entities of mature NK cell neoplasms in the current World Health Organization (WHO) Classification. One is extranodal NK cell lymphoma, nasal type (ENKL) [4], and the other is aggressive NK cell leukemia (ANKL) [5]. Both diseases were recognized in the mid-1980s, but the relationship and boundary between these two diseases remains unclear.

ENKL is an entity of non-Hodgkin's lymphoma defined in the WHO Classification, which is characterized by extensive extranodal involvement with NK or T cell [1–4]. It mostly occurs in the nasal and/or paranasal area and is called 'nasal NK/T-cell lymphoma' in this situation. This type of lymphoma is rare in Western countries but is more frequent in East Asia or in Central and South America. Its frequency among all malignant lymphomas is ~3.3% in Japan [6], 6% in Hong Kong [7], 8% in Korea [8] and 5% in Taiwan [9]. Histopathologically, the lymphoma cells are polymorphous and show an angiocentric growth pattern, which induces vascular obstruction and prominent necrosis. Tumor cells are usually NK cells (surface CD3−, cytoplasmic CD3ε+, and CD56+) in most cases [10–13] but in rare cases can also be T cells.

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Epstein–Barr virus (EBV) is found in the tumor cells in virtually all cases, and therefore ENKL is now regarded as one of the EBV-related neoplasms [14, 15].

ANKL has been recognized as a subtype of large granular lymphocyte (LGL) leukemia [16–18] and is now regarded as a distinct subtype in the WHO Classification [5]. Because of its rarity, ANKL had not been fully characterized until recently. This leukemia is characterized by systemic proliferation of NK cells, with an immunophenotype of surface CD3− CD2+ CD16+ CD56+ and germline configuration of T-cell receptor (TCR) genes. Its clinical course is highly aggressive, and most patients die within 2 years, many of them within 6 months after diagnosis [18].

For ENKL, several Asian investigators reported clinical and prognostic characteristics in a relatively large number of patients [19–21]. These reports clearly demonstrated that >80% of ENKL presents with localized disease, and the International Prognostic Index (IPI) score is a good indicator for prognosis. However, all the reports excluded ANKL from their analyses and did not mention the relationship of ENKL and ANKL. In addition to IPI, other novel prognostic factors have been identified by Korean groups: local tumor invasiveness (LTI) [20] and regional lymph

Table 1. Patient characteristics of NK cell neoplasms

	Nasal, *n* (%)	Extranasal, *n* (%)	Aggressive, *n* (%)	*P* value
Number	123	27	22	
Age (years), median (range)	52 (14–89)	57 (23–89)	42 (12–80)	0.04
Sex (male/female)	81/42	12/15	7/15	0.003
Stage				<0.0001
I	55 (45)	6 (22)	0	
II	29 (24)	4 (15)	0	
III	8 (7)	1 (4)	0	
IV	31 (25)	16 (59)	22 (100)	
B symptom	56 (46)	17 (63)	18 (82)	0.004
Performance status				0.0005
0	49 (40)	6 (22)	2 (10)	
1	49 (40)	10 (37)	9 (43)	
2	11 (9)	4 (15)	3 (14)	
3	8 (7)	2 (7)	2 (10)	
4	5 (4)	5 (19)	5 (24)	
Sites of involvement				
Lymph node	31 (25)	11 (41)	9 (41)	0.13
Extranodal	123 (100)	26 (96)	22 (100)	
Nose	111 (90)	0	0	<0.0001
Pharynx	28 (23)	1 (4)	1 (5)	0.01
Skin	19 (15)	17 (63)	3 (14)	<0.0001
Liver	10 (8)	10 (37)	14 (64)	<0.0001
Spleen	10 (8)	11 (41)	12 (55)	<0.0001
Lung	10 (8)	3 (11)	2 (10)	0.88
Central nervous system	7 (6)	2 (7)	1 (5)	0.91
Bone marrow	9 (7)	9 (33)	22 (100)	<0.0001
Peripheral blood	3 (2)	1 (4)	19 (86)	<0.0001
Others	10 (8)	5 (19)	3 (14)	0.24
International Prognostic Index				0.0001
Low	58 (47)	5 (19)	0	
Low–intermediate	34 (28)	8 (30)	3 (14)	
High–intermediate	13 (11)	4 (15)	9 (41)	
High	18 (15)	10 (37)	10 (46)	
Prognostic index for T-cell lymphoma				0.0001
Group 1	45 (37)	2 (7)	0	
Group 2	43 (35)	8 (30)	1 (5)	
Group 3	27 (22)	10 (37)	10 (46)	
Group 4	8 (7)	7 (26)	11 (50)	
Korean index for NK/T-cell lymphoma				0.0001
Group 1	37 (30)	2 (7)	0	
Group 2	30 (24)	6 (22)	2 (9)	
Group 3	29 (24)	5 (19)	2 (9)	
Group 4	27 (22)	14 (52)	18 (82)	

NK, natural killer.

node involvement [21]. The latter group further proposed a novel prognostic scoring method including regional lymph node involvement, clinical stage, presence of B symptoms and serum lactate dehydrogenase (LDH) level.

In this article, the NK-cell Tumor Study Group analyzed two mature NK cell neoplasms, ENKL and ANKL, and investigated prognostic factors.

patients and methods

diagnosis

The diagnosis of ENKL was made according to both histologic and immunophenotypic characteristics as described in the WHO Classification of 'Tumours of Haematopoietic and Lymphoid Tissues' [4, 5]. Patients were diagnosed with ENKL when the tumor cells from the biopsy specimen showed infiltration of pleomorphic tumor cells with cytoplasmic CD3+ and CD56+ phenotype and the presence of EBV. Several exceptional cases that lacked only one immunophenotypic marker or EBV but were also accompanied by other typical ENKL features were included for analysis. These atypical cases required germline configuration of TCR genes. For patients with typical phenotype, TCR studies were not mandatory. Angiocentricity and necrosis were not required for diagnosis but were used to help diagnosis. Those transformed from chronic active EBV lymphoproliferative disorder or chronic NK cell lymphocytosis were excluded, as were those diagnosed with blastic NK cell lymphoma (precursor NK cell lymphoblastic leukemia/lymphoma and blastic plasmacytoid dendritic cell neoplasm) [22] or any other types of T-cell lymphoma.

The diagnosis of ANKL was made as previously described [18]. Briefly, patients were diagnosed with ANKL when a proliferation of LGLs of NK cell phenotype was found in the peripheral blood (PB) and/or bone marrow (BM) exceeding 30% of the total nuclear cells. To exclude T-cell type LGL leukemia and leukemic infiltration of other T-cell lymphomas, the tumor cells were required to be negative for surface CD3 as determined by flow cytometry or germline configurations of TCR genes. The presence of EBV was determined with either *in situ* hybridization with an Epstein–Barr-encoded RNA probe or with Southern blotting but was not required for the diagnosis. Complete response (CR) is defined as complete disappearance of tumors and all objective signs of disease lasting at least 4 weeks. Partial response (PR) decrease at least 50% reduction of the tumor at least 4 weeks without occurrence of new lesions.

Table 2. Phenotypic characteristics of NK cell neoplasms

	Nasal (N = 123)	Extranasal (N = 27)	Aggressive (N = 22)	P value
CD1	0/20	0/4	0/12	NS
CD2	49/58 (84%)	18/20 (90%)	22/22 (100%)	0.14
CD3	0/53	0/15	0/22	NS
CD4	2/88 (2%)	1/20 (5%)	0/22	0.56
CD5	4/85 (5%)	0/16	0/17	0.45
CD7	22/34 (65%)	3/6 (50%)	14/19 (74%)	0.55
CD8	12/88 (14%)	6/20 (30%)	6/21 (29%)	0.10
CD10	1/20 (5%)	0/5	0/14	0.61
CD11b	2/5 (40%)	0/3	2/7 (29%)	0.46
CD13	0/11	0/5	0/17	NS
CD14	0/10	0/4	0/8	NS
CD15	0/8	0/6	0/5	NS
CD16	9/40 (23%)	2/9 (22%)	15/20 (75%)	<0.001
CD19	1/38 (3%)	0/10	0/17	NS
CD20	1/68 (1%)	0/17	0/15	NS
CD25	0/7	0/8	0/17	NS
CD33	1/14 (7%)	0/4	0/14	NS
CD34	0/5	0/2	0/15	NS
CD56	115/120 (96%)	26/27 (96%)	21/21 (100%)	0.64
CD57	2/41 (5%)	0/9	2/16 (13%)	0.40
HLA-DR	21/24 (88%)	6/7 (86%)	16/16 (100%)	0.32
TCRαβ	0/16	0/10	0/11	NS
TCRγδ	0/16	0/7	0/10	NS
cyCD3	68/86 (79%)	21/22 (95%)	3/7 (43%)	0.009
TdT	0/2	0/1	0/5	NS
CD30	3/7 (43%)	1/4 (25%)	ND	NS
CD43	15/17 (88%)	3/3 (100%)	ND	NS
CD45RO	44/49 (90%)	8/10 (80%)	ND	NS
CD79a	0/30	0/7	ND	NS
TIA-1	62/65 (95%)	16/16 (100%)	2/2 (100%)	NS
Granzyme B	55/57 (96%)	13/15 (87%)	2/2 (100%)	NS
EBV	93/94 (99%)	24/26 (92%)	11/13 (85%)	NS

HLA-DR, human leukocyte antigen; NK, natural killer; NS, not significant; cyCD3, cytoplasmic CD3; TdT, terminal deoxynucleotidyl transferase; ND, not determined; EBV, Epstein–Barr virus.

patients

A total of 172 mature NK cell leukemia/lymphomas (150 ENKLs and 22 ANKLs) were included in this study. Detailed clinicopathologic characteristics of 22 ANKLs were described previously [18]. This study was conducted by the NK-cell Tumor Study Group and approved by the institutional review board of participating institutions. Initial diagnosis was made at each institution and was revised when the original histologic material was available. Specimens were reviewed by two expert hematopathologists (SN and JS) and clinical data were reviewed by the Diagnostic Committee (RS, KK and KO) as described previously [23].

histologic and immunophenotypic examination

Histopathologic examination was conducted on formalin-fixed and paraffin-embedded sections of tissue after staining with hematoxylin–eosin. Immunohistochemical studies for various antigens and *in situ* hybridization for EBV-encoded RNA was carried out as described [24]. Briefly, the expression of antigens in the paraffin-embedded sections was examined by using the avidin–biotin complex peroxidase method. The antibodies comprised CD3 (Dako; Santa Fe, CA), CD56 (Novocastra Laboratories; Newcastle, UK), L26/CD20 (Dako), CD79a (Dako), UCHL1/CD45RO (Dako), MT1/CD43 (Bio-Science Products; Emmenbrucke, Switzerland), CD4 (Novocastra), CD8 (Dako), E29/EMA (Coulter Immunology; Hialeah, FL), Leu7/CD57 (Becton-Dickinson; Sunnyvale, CA), LMP-1 (Dako), DO-7/p53 (Dako), bcl-2 (Dako), TIA-1 (Coulter Immunology) and granzyme B (Monosan; Uden, The Netherlands). Flow cytometric immunophenotyping including cytoplasmic CD3 (cyCD3) was carried out, as described previously [25]. For CD16 antibody, Leu11 (Becton-Dickinson; Franklin Lakes, NJ) and ION16 (Beckman Coulter; Fullerton, CA) were used.

statistical analysis

The LDH index was calculated at each of the institutes from a patient's serum LDH level divided by the upper limit of serum LDH. IPI and prognostic index for T-cell lymphoma (PIT) scores were calculated as previously described [26, 27]. The treatment response was assessed according to standard response criteria [28]. Overall survival (OS) was measured from the date of diagnosis to the date of death or the last follow-up. Correlations between the two groups were examined with the χ^2 test, Fisher's exact test and the Mann–Whitney U test. Patient survival data were analyzed with the method of Kaplan and Meier and were compared by means of the log-rank test. Univariate and multivariate analyses were carried out using the Cox proportional hazards regression model, and variables were selected with the stepwise method. Data were analyzed with STATA version 9 (Stata Corporation, College Station, TX) and Fisher (Nakayama-Shoten; Tokyo, Japan) statistical software.

results

patient characteristics

The characteristics of 172 patients are listed in Table 1. Of the 150 ENKLs, 123 presented with nasal and/or paranasal lesions, which were categorized as nasal NK cell lymphoma in the following analyses. Remaining 27 did not show any nasal/paranasal involvements by physical examination and computer tomography and were categorized as extranasal NK cell lymphoma. The origin of these cases was the skin in 16 subjects, the liver and/or spleen in 10, and the intestine in 1. ANKL showed significantly younger age onset than ENKL (median: 42 versus 53 years, $P = 0.04$). Nasal ENKL showed male predominance (male : female, 81 : 42), but extranasal ENKL (12 : 15) and ANKL (7 : 15) did not ($P = 0.003$). Among ENKL cases, those originating from the nasal region showed a higher percentage of localized disease (stage I) than those from extranasal sites (45% versus 22%, $P = 0.03$). The former

Table 3. Therapy and response

	Number of patients					CR rate (%)	Response rate (%)
	CR	PR	NR	UE	Total		
Stage I	44	5	9	3	61	73	82
Nasal: chemotherapy alone	11	2	3	1	17	65	76
Nasal: radiotherapy alone	5	0	1	0	6	83	83
Nasal: chemotherapy followed by radiotherapy	13	1	4	0	18	72	78
Nasal: radiotherapy followed by chemotherapy	9	1	1	0	11	82	91
Nasal: concurrent chemoradiotherapy	1	0	0	0	1	100	100
Extranasal	5	1	0	0	6	83	100
Stage II	13	11	9	0	33	39	73
Nasal	11	9	9	0	29	38	69
Extranasal	2	2	0	0	4	50	100
Stage III	5	1	2	1	9	56	67
Nasal	4	1	2	1	8	50	63
Extranasal	1	0	0	0	1	100	100
Stage IV	7	10	25	5	47	15	36
Nasal	5	8	16	2	31	16	42
Extranasal	2	2	9	3	16	13	25
Aggressive NK cell leukemia	4	3	12	3	22	18	32

Two of the nasal ENKLs with stage I disease did not receive any therapy due to a poor condition.
CR, complete response; PR, partial response; NR, no response; UE, unevaluable.

showed a significantly lower distribution of the clinical stage than the latter ($P = 0.002$). The presence of B symptoms was high in extranasal ENKL and ANKL but low in nasal ENKL ($P = 0.004$). The performance status of patients with extranasal origin was significantly worse than that of patients with nasal origin ($P = 0.01$). All but one case showed extranodal involvement at initial presentation. Of the 123 cases of nasal NK cell lymphoma, 111 presented with involvement of the nasal/paranasal sinus, 28 with the pharynx/oral cavity and 16 with both. Only one case presented with systemic lymph node involvement. Distributions of all three prognostic indexes were significantly lower for the nasal ENKL group.

immunophenotype

Results of immunophenotyping are summarized in Table 2. Most cases of each type were positive for CD2, CD56, human leukocyte antigen-DR, TIA-1 and granzyme B, indicating their NK cell origin. CD7, CD8, CD16, CD57 and cyCD3 were positive in varying degrees. Those examined were proved to be uniformly negative for T- (CD1, CD3, CD4, CD5 and TCRs), B- (CD10, CD19, CD20 and CD79) and myelomonocytic markers (CD13, CD14, CD15 and CD33), as well as for CD25 and CD34. The expression of cyCD3 was significantly higher for ENKL (82% versus 43%, $P = 0.009$) but that of CD16 was lower (22% versus 75%, $P < 0.001$) than for ANKL. Epstein–Barr virus was also detected in most of the cases.

therapy and clinical course

Of 55 patients with stage I nasal ENKL, 17 received radiotherapy first and 35 chemotherapy first. Eleven of the former were further treated with supplemental chemotherapy, and 18 of the latter received additional radiotherapy. One patient was treated with simultaneous chemoradiotherapy, but two could not be treated due to their poor condition. Twenty-five patients received hematopoietic stem-cell transplantations (HSCTs; 17 autografts and 8 allografts), which were described previously [29, 30]. For stage I patients, complete response (CR) rate was 73%, and the response rate was 82% (Table 3). Although no statistical superiority was found, the CR rate exceeded 80% for patients who received radiotherapy first. In contrast, the CR rates for patients treated with chemotherapy alone and those with chemotherapy followed by radiotherapy were 65% and 72%, respectively. All but three patients with stage II disease received chemotherapy. Two of the patients received radiotherapy, but one could not receive any therapy due to the poor condition. Each two of the patients with stage III and IV disease could not also receive any therapy, but the others were treated with various types of combination chemotherapy. The CR rate was significantly different by the clinical stage (39% in stage II, 56% in stage III and 15% in stage IV). No significant differences in response rates were found between nasal and extranasal ENKLs when stratified by clinical stage. The CR and response rates of stage IV patients were comparable with those of ANKL.

difference between ANKL and ENKL

The OS curves of nasal and extranasal ENKL and ANKL are shown in Figure 1A. Prognosis was significantly different

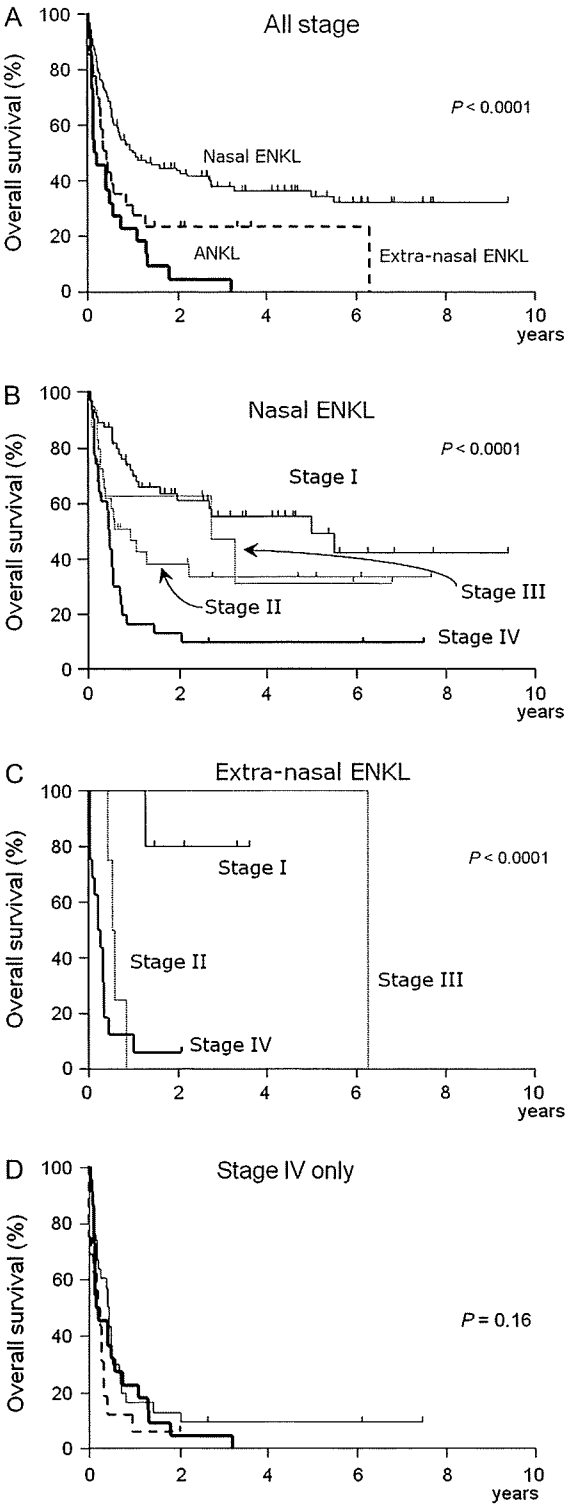

Figure 1. Overall survival (OS) of patients with aggressive NK cell leukemia (ANKL) and extranodal NK cell lymphoma (ENKL) according to clinical stage. (**A**) Prognosis was different among ANKL (thick line), nasal ENKL (thin line) and extranasal ENKL (broken line). Four-year OS was 36% for nasal ENKL and 23% for extranasal ENKL. (**B**) Nasal ENKL shows different prognosis according to the clinical stage ($P < 0.0001$). Four-year OS was 55% for stage I, 33% for stage II, 31% for stage III and 10% for stage IV patients. (**C**) Extranasal ENKL also shows different prognosis according to the clinical stage ($P = 0.001$). (**D**) If restricted to stage IV patients, no significant difference in OS was found ($P = 0.16$).

Table 4. Prognostic factors affecting overall survival

Variables	Unfavorable factors	Univariate		Multivariate[a]	
		Hazard ratio (CI)	*P*	Hazard ratio (CI)	*P*
Age (years)	>60	1.1 (0.8–1.6)	0.59	–	
Stage	III/IV	3.2 (2.2–4.7)	<0.000001	1.7 (1.0–2.8)	0.04
PS	2–4	3.0 (2.0–4.4)	0.000001	1.9 (1.2–2.9)	0.003
Extranodal disease	More than one site	3.0 (2.1–4.5)	<0.000001	1.8 (1.1–3.1)	0.03
LDH	Above normal	2.1 (1.5–3.1)	0.00008	–	
B symptom	Present	2.2 (1.5–3.3)	0.00002	–	
Bone marrow	Involved	2.7 (1.8–4.0)	0.000001	–	
Regional LN	Involved	1.5 (1.1–2.3)	0.03	–	
Disease type	Extranasal/aggressive	2.3 (1.6–3.4)	0.00002	1.6 (1.1–2.5)	0.02
WBC count	>10 000/mm^3	1.8 (1.0–3.3)	0.05	–	
IPI category	H-I/H	3.6 (2.5–5.2)	<0.000001		
PIT category	Group 3/4	2.7 (1.9–4.0)	0.000001		
Korean index	Group 3/4	2.9 (2.0–4.3)	0.000001		

[a]Final model.

CI, confidence interval; PS, performance status; LDH, lactate dehydrogenase; LN, lymph node; WBC, white blood cell; IPI, International Prognostic Index; H-I, high–intermediate; H, high; PIT, prognostic index for T-cell lymphoma.

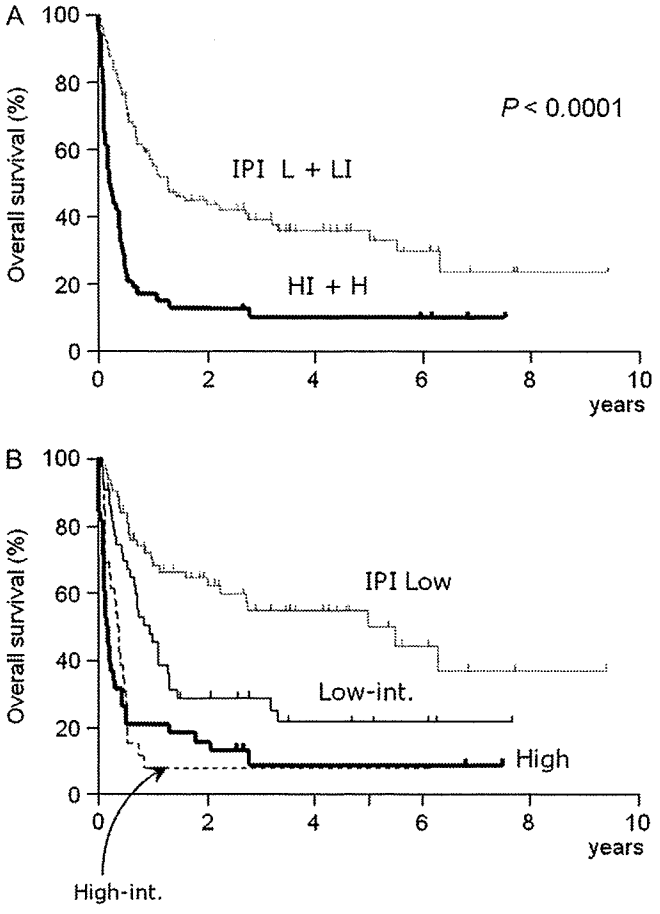

Figure 2. Overall survival of natural killer (NK) cell malignancies according to the International Prognostic Index (IPI). (**A**) Patients with high–intermediate/high IPI score showed significantly lower survival ($P < 0.0001$). (**B**) However, prognosis was almost the same for those with high–intermediate and high-risk categories.

among the three disease categories ($P < 0.0001$). The OS curves of nasal and extranasal ENKL with different clinical stages are shown in Figure 1B and C, respectively. Prognosis was significantly different according to the clinical stage ($P < 0.0001$), but stage III group showed better prognosis than stage II group. If restricted to patients with stage IV diseases, the prognosis was equally poor for all disease subtypes (Figure 1D, $P = 0.16$). Due to this result and the similar CR rates between ANKL and stage IV ENKL noted above, we compared the clinicopathologic characteristics of ANKL and stage IV ENKL (supplemental Table S1, available at *Annals of Oncology* online). Although differences in age of onset, presence of B symptoms, performance status and IPI vanished when restricted to stage IV cases, sex distribution and phenotypic markers (CD16 and cyCD3) were still significantly different. For stage IV ENKL cases, male : female ratio was 31 : 16, and CD16 and cyCD3 were positive in 5 of 18 (28%) and 27 of 33 cases (82%), respectively.

prognostic factors and model

Although patient age was not a significant prognostic factor, univariate Cox analysis identified the following prognostic factors: clinical stage, performance status, number of extranodal involvements, serum LDH index, presence of B symptoms, BM involvement, regional lymph node involvement, disease type and white blood cell count (Table 4 and supplemental Figure S1, available at *Annals of Oncology* online). IPI category, PIT and Korean index were also highly prognostic (Figures 2 and 3). Multivariate analysis revealed four factors, advanced stage (III or IV), poor Eastern Cooperative Oncology Group performance status (2–4), extranodal involvement (more than one) and disease type (extranasal/aggressive), to be significant and independent prognostic factors (Table 4). All patients were then scored according to these four factors. Fifty-eight patients did not have any of the factors, 29 had one factor, 37 had two factors, 29 had

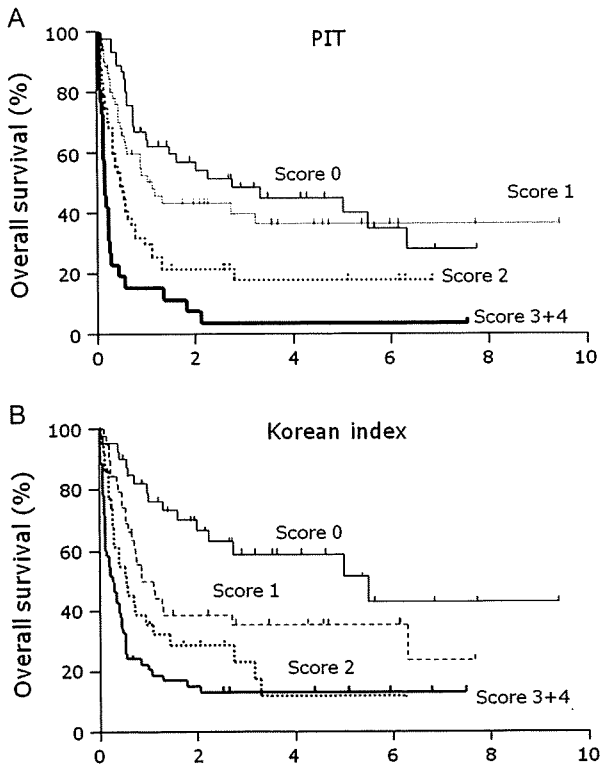

Figure 3. Overall survival of natural killer cell malignancies according to the prognostic index for T-cell lymphoma (PIT) and the Korean prognostic index. (**A**) Although the PIT score could successfully identify patients with poor prognosis, the differentiation between those with score 0 and score 1 was not clear enough. (**B**) Although the Korean index could successfully identify patients with poor prognosis, the distinction between those with score 2 and score 3 + 4 was not intelligible.

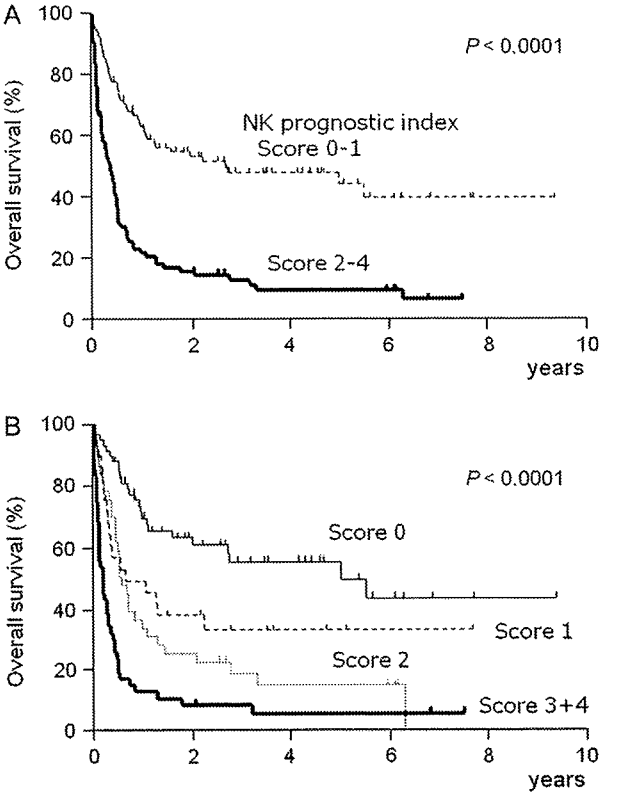

Figure 4. Overall survival of natural killer (NK) cell malignancies according to new NK prognostic index. Patients are successfully stratified according to the new prognostic index comprising clinical stage, performance status, number of extranodal involvements and disease type, either two (**A**) or four subgroups (**B**).

three factors and 18 had all four factors. The OS curves according to the new NK cell tumor prognostic index are shown in Figure 4. The new index categorized four groups with significantly different prognoses. Four-year OS rate was 48% for patients with score 0 or 1 and 11% for those with score 2–4 (Figure 4A, $P < 0.0001$). Four-year OS rates were 55%, 33%, 15% and 6% for patients with score 0, score 1, score 2 and score 3 or 4, respectively (Figure 4B, $P < 0.0001$).

discussion

Since the recognition of ENKL and ANKL 20 years ago, precise comparisons have not yet been satisfactorily conducted. Although the clinicopathologic characteristics of nasal NK cell lymphoma and ANKL are very different, the immunophenotypic profiles, genotype (germline TCR genes) and the close association with EBV are quite similar. Particularly, ENKL of extranasal origin shows high incidence of BM involvement and aggressive clinical course [31–36]. On the other hand, ANKL is characterized by a predilection to hepatosplenic involvement, indicating the existence of a spectrum between these two diseases [37]. However, our analysis showed discrete differences for the age of onset and expression of CD16. Although difference in cyCD3 expression was recognized, only a few numbers of cases were examined. The difference of cyCD3 expression should be examined in a large number of cases. For extranasal NK cell lymphomas, the skin was the most common site of involvement but not for ANKL. Considered together with the difference in genomic gain/loss profiles [38, 39], ANKL and ENKL are concluded to be different in several disease features. However, no specific genes or regions have been identified to separate these two diseases. We tentatively set the boundary of ANKL and ENKL as 30% of BM/PB involvement because this is the most objective criterion [18]. If this differentiation is not generalized, the boundary of ANKL and ENKL can be ambiguous. Further investigations are needed to identify the biological differences between the two diseases. For the consideration of clinical management, therapeutic responses and prognoses of ANKL and stage IV ENKL are similarly poor, indicating that both diseases should be treated equally.

Long-term survival of stage I cases from our series was ~40%. This is consistent with other studies for NK cell lymphoma in the literature [40–43] but lower than that of other lymphoma subtypes [44]. One reason for the poor prognosis of this lymphoma is the expression of P-glycoprotein, which mediates multidrug resistance [45, 46]. Therefore, radiotherapy plays a key role in the treatment of this lymphoma. We compared response rates and prognoses with the initial therapeutic strategy. Patients who were treated with radiotherapy followed by chemotherapy showed the most

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favorable response and prognosis, but the differences were not statistically significant. Currently, involved-field radiotherapy followed by chemotherapy is still regarded as a standard treatment of early-stage diseases [47]. Simultaneous chemoradiotherapy is now under evaluation by a Japanese group [48].

For advanced-stage cases, although long-term survival after high-dose chemotherapy and HSCT has been reported [29, 49–51], no standard chemotherapy is currently available [52]. Because L-asparaginase has been reported to be effective in several patients with NK/T-cell lymphoma [53–55], we recently conducted a phase I study of combination chemotherapy including L-asparaginase [56]. The SMILE regimen comprises a steroid (dexamethasone), methotrexate, ifosfamide, L-asparaginase and etoposide. Methotrexate, ifosfamide and L-asparaginase are multidrug resistance-unrelated agents and etoposide shows both *in vitro* and *in vivo* efficacy for EBV-associated lymphoproliferative disorders. Level 1 SMILE was feasible and the overall response rate was 67% [56]. The SMILE regimen is a promising combination chemotherapy for advanced stage of ENKL and ANKL.

The current study identified that the IPI category was also prognostic for extranodal NK/T-cell lymphoma, which is consistent with other studies in the literature [19–21]. However, regarding the IPI components, only age was not prognostic, in contrast to other factors (clinical stage, serum LDH level, performance status and number of extranodal sites). Absence of age as a prognostic factor is consistent with the Korean study, which included the largest number of patients [21]. The Korean study also identified a novel prognostic index including B symptoms, clinical stage, serum LDH level and regional lymph node involvement. This Korean index [21], as well as the PIT category [27], was also prognostic for our series of patients, but multivariate analysis identified another combination of factors that was the most prognostic for patients of the current study. Another Korean group pointed out LTI as a significant prognostic factor [20]. In our patients, however, LTI was only recognized in a limited population, and therefore, LTI was not a significant prognostic factor. Notably, in our series, extranasal onset of disease has been identified as one of the significant prognostic factors. For ENKL, the difference between nasal and extranasal origin is a focus of interest in the recently published result of International Peripheral T/NK cell Lymphoma Project [57, 58]. Further investigations are needed to identify the appropriate prognostic model for extranodal NK cell lymphoma and leukemia.

In conclusion, stage IV ENKL and ANKL are different in several clinicopathologic features but show similar therapeutic response and prognosis. Our novel NK prognostic index is useful for improving treatment choices and designing clinical trials to evaluate new treatment strategies.

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disclosure

KO is currently an employee of Eisai Pharmaceutical Company.

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