NK-Cell Neoplasms

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Natural killer (NK) cell-derived neoplasms were first reported in the late 1980s, and the definition of this disease was established in the 1990s [1]. NK-cell neoplasms mainly arise in East Asia and Latin America and usually show an aggressive clinical course. In the 2000s, detailed clinicopathologic features and effective therapeutic approaches have been actively investigated. In this chapter, we provide an overview of the clinicopathologic and molecular features of NK-cell neoplasms and summarize the recent progress in the treatment of these diseases.

Definition, Subtypes, and Clinical Characteristics

Definition and Subtypes of NK-Cell Neoplasms in the 2008 WHO Classification

The 2008 World Health Organization (WHO) classification recognizes two NK-cell neoplasm categories: extranodal NK/T-cell lymphoma, nasal type (ENKL), and aggressive NK-cell leukemia (ANKL) [2]. The former edition of the WHO classification (2001) also recognized blastic NK cell lymphoma as an NK-cell neoplasm [3]. However, the cumulative evidence from detailed immunophenotyping, gene expression profiling studies [4], and characterization of blastic NK-cell lymphoma cell lines [5] strongly suggests that blastic NK cell lymphoma is derived from a plasmacytoid dendritic cell [2] and is classified as such in the 2008 WHO classification.

Definition and Subtypes of ENKL

ENKL is defined as "a predominantly extranodal lymphoma characterized by vascular damage and destruction, prominent necrosis, cytotoxic phenotype and association with the Epstein–Barr virus (EBV)" in the 2008 WHO classification [2]. It was formerly designated as lethal midline granuloma, angiocentric T-cell lymphoma, or polymorphic reticulosis. ENKL commonly occurs in the upper aerodigestive tract, with the nasal cavity being the most common site of involvement. ENKL also involves the extra-upper aerodigestive tract, such as the skin, soft tissue, gastrointestinal tract, lung, and testis [2]. In the 2001 WHO classification, two clinical subtypes of ENKL, nasal NK/T-cell lymphoma and nasal-type NK/T-cell lymphoma, were described [3]. The former was characterized by lymphomatous involvement of the nasal cavity and/or its adjacent sites. Conversely, nasal-type NK/T-cell lymphoma occurs outside the nasal cavity, such as in the skin or gastrointestinal tract [3]. There are also differences in the clinical characteristics and

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therapeutic outcomes between these two subgroups. An international survey has revealed that extranasal NK/T-cell lymphoma is more frequent in Continental Asia than in Japan and is characterized by a shorter survival compared to nasal NK/T-cell lymphoma [6]. This latter finding was confirmed by a large retrospective survey from Korea [7]. The two subgroups have been managed with considerably different treatment strategies, as will be described later in this chapter. Moreover, it is known that extensive examinations, such as random biopsy of the nasal mucosa, occasionally reveal nasal involvement in patients with non-nasal NK/T-cell lymphoma [8]. It is noteworthy that the term "nasal NK-cell lymphoma" is not used in the 2008 WHO classification, although it is widely adopted in current research and in clinical practice.

ENKL rarely presents as a primary nodal lymphoma. Nodal NK/T-cell lymphoma is characterized by frequent CD8 expression and shows clinical features between ENKL and EBV-associated cytotoxic nodal T-cell lymphoma [9]. NK/T-cell lymphoma of the Waldeyer's ring is reported to be associated with frequent cervical node involvement and relatively longer survival [10]. Few cases of intravascular large cell lymphoma of the NK-cell phenotype have been reported [11]. However, additional validating studies are needed to determine the significant differences and thus discriminate between these particular ENKL subgroups.

Definition and Overview of ANKL

ANKL has been recognized as a subtype of large granular lymphocyte (LGL) leukemia since the 1980s and is now recognized as a distinct subtype in the WHO classification [2, 12]. It is characterized by the systemic proliferation of NK cells and has a highly aggressive clinical course, although it accounts for less than 1% of the lymphoid malignancies in East Asia. The leukemic cells in ANKL show an LGL morphology, a surface $CD3^-$ $CD2^+$ $CD16^+$ $CD56^+$ immunophenotype, and germline configurations of the T-cell receptor genes [13, 14]. The tumor cells express P-glycoprotein [15, 16] and are positive for EBV, as also seen in ENKL [13]. ANKL can be differentially diagnosed from chronic lymphoproliferative disorders of NK cells [2] (or chronic NK lymphocytosis [17]) by the presence of clonal cytogenetic abnormalities and/or analysis of the terminal repeat sequence of EBV by Southern blotting. Although there have been some reports of rare cases of ANKL transformed from chronic NK lymphocytosis [18], the etiology of this lymphoma subtype is still unknown.

ANKL displays the clinical features of leukemia, which include anemia and thrombocytopenia. Occasionally, patients with ANKL experience hepatosplenomegaly and/or lymph node swelling with no leukemic cells in either the bone marrow or peripheral blood. Some investigators have speculated that ANKL may be a leukemic subtype of ENKL (ANKL/lymphoma). However, there are some differences in the properties of the tumor cells between ANKL and ENKL. For example, neoplastic cells of ANKL express CD16 more frequently than those of ENKL, whereas CD3 epsilon expression is less frequent [13]. Genomic imbalances also differ between ENKL and ANKL [19].

Patients with ANKL have been treated with chemotherapeutic regimens for acute leukemia or aggressive lymphoma, but the response and prognosis are poor. The majority of patients die within 2 years, and many die within 6 months of their diagnosis [13]. Partly due to the rarity of the disease, the detailed clinicopathologic features and optimal treatment of ANKL have not been extensively investigated.

Clinical Characteristics of ENKL

The most frequent site of involvement in ENKL is the nasal cavity and its adjacent sites, such as the paranasal sinuses, nasopharynx, palate, Waldeyer's ring, and orbit. Nasal obstruction, nasal discharge, and epistaxis are common initial symptoms. An episode of unilateral epistaxis is often the stimulus for referral to a medical center. Eye symptoms may occur when ENKL involves the orbit or its adjacent sites. Also of note, ENKL is sometimes associated with complications such as hemophagocytic syndrome or fevers of unknown origin.

Table 6.1 Summary of the clinical characteristics at diagnosis from large retrospective studies of nasal NK/T-cell lymphoma and whole ENKL

	Nasal NK/T-cell lymphoma		Whole ENKL	
Parameter	Au ($n=92$) [6]	Suzuki ($n=123$) [20]	Lee ($n=262$) [21]	Kim ($n=280$) [7]
Male sex (%)	64	66	65	66
Median age	52	52	–	46
Age >60 years (%)	–	–	21	21
Performance status >1 (%)	9	20	13	23
Stage III/IV (%)	27	32	24	25
LDH >upper normal limit (%)	45	–	37	45
Number of sites of extranodal involvement >1 (%)	16	–	–	22
B symptoms (%)	39	46	35	42
High or high-intermediate risk of IPI (%)	–	26	19	21
NK-PI Group 3/4 (%)	–	46	42	42

IPI International Prognostic Index; *NK-PI* NK/T-cell lymphoma prognostic index

Table 6.1 summarizes the clinical characteristics of nasal NK/T-cell lymphoma and whole ENKL at diagnosis, based on studies of large patient cohorts [6, 7, 20, 21]. Because the incidence of extranasal (nasal-type or non-nasal) ENKL is 25% or less among cases with this lymphoma subtype, the principal clinical data that have been assembled for nasal NK/T-cell lymphoma and whole ENKL are very similar, as shown in Table 6.1. Approximately 65% of patients with ENKL are male, and the median age at diagnosis ranges from 46 to 52 years. Seventy-five percent of ENKL patients have stage I or II disease. The serum LDH levels are elevated in 45% of patients, whereas the relative frequency of a poor performance (PS) status (>1) is low. Based on these features, ~80% of patients with ENKL are classified as low or low-intermediate risk according to the International Prognostic Index (IPI) [22]. B symptoms of the Ann Arbor classification are present in ~45% of patients, which is more frequent than other localized aggressive lymphomas.

ENKL can disseminate preferentially to the skin, gastrointestinal tract, testis, and central nervous system (CNS). The initial symptoms of ENKL with gastrointestinal involvement often include perforation of the gastrointestinal tract, which can be a serious complication during chemotherapy.

Staging of ENKL

Because ENKL usually begins at extranodal sites, the staging of this lymphoma is often problematic. In the Ann Arbor classification system, stage IE is defined as the localized involvement of a single extralymphatic organ or site in the absence of any lymph node involvement. The diffuse or disseminated involvement of one or more extralymphatic organs is defined as stage IV. However, in most reports of ENKL, contiguous involvement extending to adjacent structures, such as the nasal cavity, paranasal sinuses, nasopharynx, oral cavity, or orbit, is considered to be stage IE (Table 6.2).

Regional lymph nodes in cases of localized nasal NK/T-cell lymphoma are considered to be equivalent to those in the cervical lymph node region using the Ann Arbor classification. In this regard, the tumor, node, metastasis (TNM) scheme for cancer of the nasal cavity and paranasal sinus defines regional lymph nodes as cervical lymph nodes. Mediastinal lymph node metastases are regarded as distant metastases. Stage IIIE disease usually accounts for <10% of nasal NK/T-cell lymphomas because it typically progresses rapidly to the bone marrow or other extranodal sites.

To evaluate the involvement of the nasal cavity and its adjacent sites in cases of ENKL, magnetic resonance imaging (MRI) of the nasal cavity is

Table 6.2 Staging of nasal NK/T-cell lymphoma[a]

IE	Contiguous involvement extending to adjacent structures (nasal cavity, paranasal sinuses, nasopharynx, oral cavity, and orbit)
IIE	Primary site with cervical lymph node involvement (contiguous stage IIE) with or without involvement of other supradiaphragmatic lymph node regions Primary site with isolated involvement of head and neck sites (Waldeyer's ring, oropharynx, hypopharynx, etc.)
IIIE (rare)	Primary site with cervical lymph node involvement with infradiaphragmatic lymph node involvement and/or involvement of the spleen
IV	Primary site (without cervical lymph node involvement) with isolated distant involvement Any involvement of the liver or bone marrow

[a]Summarized for this review using data from previous reports of ENKL and the current consensus in clinical practice

thought to be more useful that a CT scan alone. ENKL is a fluorodeoxyglucose (FDG)-avid lymphoma, and the usefulness of (18)F-FDG positron emission tomography (FDG-PET) in the staging of this lymphoma has also been reported in at least four independent studies [23–26]. To assess bone marrow involvement, in situ hybridization analysis of EBV-encoded RNA (EBER) has also been shown to be useful [27]. These new staging procedures are expected to allow for the discrimination of patients with advanced-stage disease who require different treatment strategies.

Epidemiology and Etiology of ENKL

Epidemiology

ENKL is much more common in Asia and Latin America than in the United States and Europe [28, 29]. In East Asia, its relative frequency among all lymphomas is approximately 3% in Japan [30], 6% in Hong Kong, 7% in Taiwan, and 9% in Korea [17]. In contrast, the relative frequency of ENKL is <1% in western countries.

Etiology

The etiology of ENKL has not yet been clarified. Because ENKL is almost always associated with EBV [31], it is regarded as one of the EBV-associated lymphoid malignancies [1]. A possible etiologic association between air pollution and ENKL was suggested by a previous Mexican study [32]. Further, a case–control study from East Asia has also revealed an etiologic association between ENKL and life-style or environmental factors, including farming, pesticide use, or residential proximity to garbage incineration facilities [33].

Pathology, Diagnosis, and Molecular Features of ENKL

Pathology and Diagnosis

Histopathologically, ENKL is characterized by an angiocentric or angiodestructive invasion of tumor cells, the formation of necroses, and infiltration by various types of inflammatory cells [2]. No definite mass formation is found in many cases. In such instances, a biopsy including normal-looking tissues or a random biopsy to obtain diagnostic tissue samples is recommended. It is known that the nuclei in the ENKL tumor cells are often elongated [2] and display a cucumber-like shape.

There are two lineages of tumor cells in ENKL, the NK- and cytotoxic T-cell immunophenotypes. In both types, CD56 is positive in most cases. However, NK-cell markers other than CD56, such as CD16 or CD57, are rarely positive in ENKL. CD5 expression strongly suggests a T-cell type of ENKL [34]. CD20 is usually negative in ENKL, but CD20-positive cases are known to exist [35]. In the NK-cell type of ENKL, the tumor cells express cytoplasmic CD3 epsilon only, whereas both surface CD3 and cytoplasmic CD3 epsilon are expressed in the T-cell type of ENKL [36–39]. NK-cell type ENKL shows

germline configurations of the T-cell receptor genes. In addition, in situ hybridization analysis for EBER using paraffin-embedded material is a useful test, because the vast majority of these lymphomas show positive nuclear signals within the tumor cells. When CD56 is negative, both a positive EBV status and the expression of more than one cytotoxic molecule (e.g., perforin, granzyme B, or TIA-1) are required for a diagnosis of ENKL [2].

During the 1990s, the diagnosis of NK-cell neoplasms was facilitated by improvements in histopathologic diagnostic procedures. For example, anti-CD56 monoclonal antibodies that could be used for routinely processed formalin-embedded paraffin sections became available. The identification of disease-specific features, such as cytoplasmic CD3 epsilon expression and frequent detection of EBER by in situ hybridization, also contributed to a more accurate differential diagnosis from other lymphoma subtypes.

Molecular Features

ENKL lymphoma cells express the multidrug resistance (*MDR*) *1/ABCB1* gene and its product, P-glycoprotein [15, 16, 40]. This MDR phenomenon is thought to be the major reason why ENKL is resistant to CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or the CHOP-like chemotherapies that comprise mainly MDR-related agents. Immunohistochemical analyses have further revealed the prognostic significance of the expression of granzyme B-specific protease inhibitor 9 (PI9) [41], cyclooxygenase-2 (COX-2) [42], or high levels of Ki-67 in the tumor cells [6]. With respect to the EBV status, EBNA1 is expressed in the tumor cells of almost all nasal NK/T-cell lymphomas and most extranasal ENKL cases [1, 31]. Other EBNAs are not expressed, but latent membrane protein 1 is expressed in 50% of affected patients [43, 44]. Based on these data, ENKL is classified as a type II latency of an EBV infection. Sequence analysis has also identified a 30-bp deletion in LMP1 in ENKL [45].

Mutations of tumor suppressor genes have been reported in ENKL. *FAS* is mutated in 50% of patients [46], and *TP53* mutations are detectable in <50% of patients [44, 47]. Several cell lines from ENKL have also been established [12], and in vitro analyses suggest that endogenous IL-9, IL-10, and IP-10 may play important roles in ENKL cell proliferation and invasion via an autocrine mechanism [44, 48, 49]. A study that combined gene expression profiling and array-based comparative genomic hybridization analyses of ENKL revealed greater transcript levels for NK-cell and cytotoxic molecules compared with peripheral T-cell lymphoma, not otherwise specified [50]. ENKL tumor cells express more vascular biology-related genes, EBV-induced genes, and *PDGFRA* when compared to normal NK cells [50]. The Akt, JAK-STAT, NF-κB, and Wnt signaling pathways are also potentially relevant biological pathways in ENKL [50]. Del(6)(q21q25) or i(6)(p10) is the most common cytogenetic abnormality in ENKL [2]. Among the known genes at 6q21, *PRDM1* and *FOXO3* are considered to be the most likely target genes in NK-cell malignancies [50, 51].

Prognostic Factors and Prognostic Models

The IPI [22] is widely used to evaluate aggressive lymphomas. Because only 20% of patients with ENKL are classified as having an IPI score of 1 or less, most studies to date have concluded that the clinical significance of this score in ENKL is not high. However, a Hong Kong study reported that the IPI is still valid for patients with an NK-cell type ENKL [52]. A multicenter retrospective study of 262 patients with ENKL from Korea proposed a prognostic model for ENKL: the NK/T-cell lymphoma prognostic index (NK-PI) or "Korean index" [21]. Four independent prognostic factors were identified in this system that were associated with overall survival (OS) in terms of the presence of B symptoms, advanced stage, an elevated serum LDH level, and the regional lymph node involvement according to the TNM system. The clinical usefulness of NK-PI has been validated in an international cooperative study [6]. Conversely, NK-PI was found not to be a useful predictor of survival in two recent prospective studies of concurrent chemoradiotherapy (CCRT) [53, 54], which are

discussed later in this chapter. Another Korean study reported the prognostic significance of local tumor invasiveness (LTI) in localized ENKL of the upper aerodigestive tract [55]. The authors defined LTI as bony invasion or perforation/invasion of the skin based on CT and physical findings and concluded that LTI is more predictive of survival than the IPI for patients with stage IE and IIE ENKL [55]. These two large retrospective studies [21, 55] indicate that high serum LDH levels, the presence of B symptoms, and LTI are important prognostic factors for ENKL, which partly differs from other aggressive lymphomas.

ENKL is an EBV-related disease, and the peripheral blood of affected patients contains fragmented EBV-DNA. Some retrospective studies suggest that measurement of the circulating viral DNA load is useful for the diagnosis, monitoring, and prognostication of this disease [56, 57]. The NK-Cell Tumor Study Group (NKTSG) conducted the first prospective study of EBV-DNA in ENKL using quantitative PCR [58]. A total of 33 patients were analyzed, and a significant correlation between the number of mononuclear cells and plasma EBV-DNA copy number was observed. The pretreatment plasma EBV-DNA levels were thus well correlated with several clinical parameters. Multivariate analysis showed that the clinical stage and pretreatment plasma EBV-DNA levels are significant prognostic factors. The plasma EBV-DNA copy number was found to be a valid indicator for both the response to treatment and OS [58]. Monitoring of EBV-DNA in the peripheral blood is already conducted routinely in some centers in East Asia [59].

Treatment of ENKL

Treatment of Localized Nasal NK/T-Cell Lymphoma

Background

Despite recent improvements in diagnostics, no standard therapy for ENKL has been established, and prior to 2009, no well-designed prospective trial for localized nasal NK/T-cell lymphoma had been reported. The 5-year OS rates for localized nasal NK/T-cell lymphoma in early reports ranged from 14 to 100% [60]. Although more than two-thirds of patients with nasal NK/T-cell lymphoma present with localized disease, their prognosis is poorer than that for individuals with a localized diffuse large B-cell lymphoma.

Most patients with localized nasal NK/T-cell lymphoma have been treated with radiotherapy with or without chemotherapy. However, the details of such treatments in many previous reports, particularly the timing of the radiotherapy and chemotherapy, have not been adequately described. Many early studies did not evaluate lymphomas arising in the nasal cavity and paranasal sinuses separately because it was believed that these were the same condition. In addition, "true" NK-cell, T-cell, and B-cell lymphomas were often intermingled in single studies due to the difficulties in immunophenotyping. Hence, the outcomes of therapeutic strategies specific for nasal NK-cell lymphoma have not been thoroughly examined to date. In this review, we focus on well-documented reports of each modality and the immunophenotyping of tumor cells.

Chemotherapy Followed by Radiotherapy

Anthracycline-containing chemotherapy followed by radiotherapy has been established as the standard treatment for localized aggressive lymphomas, mainly diffuse large B-cell lymphomas [61]. However, this therapeutic strategy is ineffective against localized nasal NK/T-cell lymphoma. The five-year OS rates of patients with localized diseases who were treated with chemotherapy followed by radiotherapy are <50% (Table 6.3) [41, 60, 62–68]. In these retrospective studies, various chemotherapeutic regimens were used. Kim et al. [69] conducted two clinical studies of the efficacy of CHOP chemotherapy for localized nasal NK/T-cell lymphoma [69]. Between 1995 and 1999, 17 patients were treated with 4 courses of CHOP chemotherapy followed by 45 Gy radiotherapy as a planned treatment. The planned sequential chemoradiotherapy could only be completed in six patients because of early disease progression during the chemotherapy phase. Subsequently, 17 patients

Table 6.3 Survival outcomes for patients with a localized nasal NK/T-cell lymphoma treated with conventional standard therapy for localized aggressive non-Hodgkin lymphomas

Modality	Stage	*n*	5 year-OS rate (%)	Reference
Cx→RT	I–II	7	14	Yu et al. [62]
Cx (→RT)	I	18	28	Kwong et al. [63]
Cx (→RT)	I/II	12	<50	Ribrag et al. [64]
Cx (→RT)	I/II	7	14	Yamaguchi et al. [60]
Cx (→RT)	I–II	61	40	Cheung et al. [65]
Cx	I–II	18	15	Li et al. [66]
Cx (→RT)	I–II	40	29	You et al. [67]
Cx (→RT)	I–IV	26	18	Pagano et al. [68]
Cx (→RT)	I–IV	48	49	Bossard et al. [41]

Cx CHOP(-like) chemotherapy; *RT* radiotherapy; *OS* overall survival

were treated more aggressively with intensified doses, dose dense CHOP and radiotherapy [70]. The complete response (CR) rate was improved, but five patients still experienced systemic failure. These results suggest that the efficacy of CHOP chemotherapy is not sufficient to treat ENKL, even at the maximal dose intensity. As previously discussed in this chapter, it has been speculated that CHOP(-like) chemotherapy comprises MDR-related agents (e.g., vincristine and doxorubicin, among others) is not effective against this disease, because ENKL tumor cells express P-glycoprotein.

EPOCH chemotherapy (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin) was originally designed to overcome the MDR phenomenon by using continuous infusion of anticancer agents. In a retrospective study from China in which 76% of a 34-patient cohort had localized disease, EPOCH chemotherapy followed by radiotherapy produced CR and 3-year OS rates of 75% [71]. However, these favorable results must be further validated in a prospective trial setting before incorporating this treatment method into clinical practice.

Frontline Radiotherapy

Radiotherapy has frequently been used as the primary treatment for ENKL, because most patients present with localized disease. In patients treated with radiotherapy alone, the CR rate is >65% [72, 73], which is the best reported single modality outcome for this disease. The reported 5-year OS rates range from 42 to 83%, but this may be caused by several selection biases, that is, patients with a small tumor burden, no systemic symptoms, or no immunophenotypic or EBV data. Kim et al. previously examined the patterns of failure in 92 patients treated with radiotherapy alone at a median dose of 50 Gy (range, 40–60 Gy) [72]. After completion of this treatment, 61 patients (66%) achieved CR, and 16 (17%) showed a partial response (PR). However, ~50% of the patients ultimately displayed a local recurrence, and systemic failure was observed in 25% of cases. In addition, all patients with systemic failure (except one) died within 1 year [72]. The OS curve declined soon after diagnosis and reached a plateau at 2 years after diagnosis. The 5-year OS rate was 40%, and the 2-year OS rate was 45%. Hence, because both in-field and systemic relapses are frequent, radiotherapy alone is not sufficient to achieve a high cure rate for patients with localized nasal NK/T-cell lymphoma.

Radiotherapy for Localized Nasal NK/T-Cell Lymphoma

To determine the optimal radiotherapy and to achieve good local control of NK/T-cell lymphoma cases, radiation oncologists in East Asia have conducted several multicenter cooperative studies over the past decade. In terms of the radiation dose, these studies have suggested that >46 Gy is needed to obtain good local control [74, 75]. It is noteworthy that ENKL tumor cells are more resistant to radiotherapy than aggressive B-cell lymphomas. In terms of radiation volume, extension to include entire nasal cavities and sinuses is reported to be more effective than simply targeting the tumor volume with a small margin [76]. Moreover, CT- or MRI-based radiation planning is also recommended to achieve good local control [75]. However, it has been shown that late adverse events in the CNS escalate in patients who receive a total dose of >60 Gy. The optimal total dose is therefore likely to be 50 Gy. It must also be considered that there are several organs adjacent to the nasal cavity, such as the optic nerve, brain stem, and retina, that are at risk

from radiotherapy. To minimize adverse events in these organs, treatments should be carefully planned using CT scanning and/or MRI by experienced radiation oncologists.

Intensity-modulated radiation therapy (IMRT) is expected to improve local control and reduce local toxicity in the treatment of ENKL. In western countries, IMRT has already been introduced as a routine treatment for localized nasal NK/T-cell lymphoma, at least in major centers. In East Asia, an attempt to substitute three-dimensional conformal radiotherapy with IMRT is ongoing [77], but conventional radiotherapy is still widely used in most institutes.

To prevent direct invasion of the brain, a sufficient margin to the side of the CNS should be established. There is also no firm evidence of any prophylactic benefit from the intrathecal administration of chemotherapeutic agents for localized nasal NK/T-cell lymphoma [78].

Frontline Radiotherapy Followed by Chemotherapy

Patients undergoing radiotherapy followed by chemotherapy have been reported to have a better prognosis [64, 79]. However, these results must be carefully evaluated because retrospective analyses may contain selection biases, and immunophenotypic analysis and the examination of the EBV status of the tumor cells were incomplete in many cases. It is also noteworthy that these earlier retrospective studies used anthracycline-containing chemotherapeutic regimens.

Concurrent Chemoradiotherapy

Overview

CCRT is expected to improve both local and systemic disease control and has been established as a standard therapy for several solid cancers. Conversely, this is not a standard treatment for lymphoma due to the generally good response of lymphomas to both chemotherapy and radiotherapy. In an earlier report from Mie University Hospital in Japan, two patients with localized nasal NK/T-cell lymphoma were successfully treated using CCRT [60]. DeVIC chemotherapy, which is a salvage chemotherapeutic regimen developed in Japan for relapsed or refractory aggressive lymphomas, was selected for concurrent use in these cases [80]. This therapy comprises dexamethasone, two MDR non-related chemotherapeutic agents (ifosfamide and carboplatin), and etoposide. Etoposide has been demonstrated to have both in vitro and in vivo efficacy against NK-cell neoplasms [81, 82] and is effective for both pediatric EBV-related hemophagocytic syndrome and pediatric EBV-associated lymphoproliferative disease [83]. The two patients [60] showed a high serum LDH level or B symptoms, which are known to be unfavorable prognostic factors in nasal NK/T-cell lymphoma [60]. However, they achieved CR and long-term survival, suggesting the potential efficacy of this treatment in high-risk patients. A Hong Kong study also reported a good therapeutic result from the use of frontline chemotherapy followed by CCRT with cisplatin and consolidative high-dose chemotherapy with autologous stem cell transplantation [84].

Because CCRT is a novel treatment modality for lymphomas, its toxicity and efficacy should be carefully evaluated in a prospective trial setting. Only one small phase II study of the use of CCRT in relapsed or refractory lymphomas with bulky disease was reported prior to 2006 [85]. In this study, radiotherapy at a median dose of 40 Gy and chemotherapy with cisplatin and etoposide were administrated concurrently. Because grade 3 or 4 hematologic toxicities were observed in 70% of the patients (14/21), the researchers concluded that hematologic toxicities and infection must be carefully managed when using this regimen.

A Prospective Trial for CCRT in a Japanese ENKL Cohort

To develop a more effective therapeutic strategy for newly diagnosed, localized nasal NK/T-cell lymphoma, the Lymphoma Study Group of the Japan Clinical Oncology Group (JCOG-LSG) conducted a phase I/II study to evaluate the

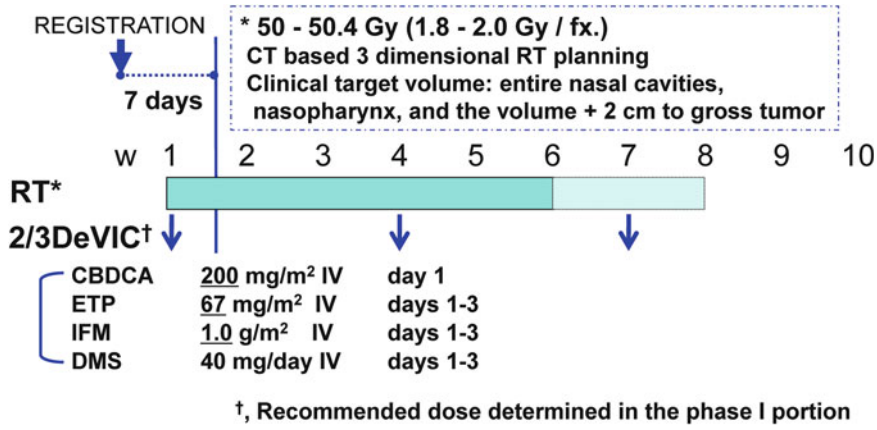

Fig. 6.1 Treatment protocol used in the JCOG0211 study. Radiotherapy at a 50 Gy dose and three courses of DeVIC chemotherapy were simultaneously initiated within 7 days of enrollment in the trial. Three dimensional conformal radiation planning using a CT scanner was performed for all patients. The clinical target volume included the entire nasal cavity, the nasopharynx, and the gross tumor volume with a margin of at least 2 cm. *RT* radiotherapy; *CBDCA* carboplatin; *ETP* etoposide; *IFM* ifosfamide; *DMS* dexamethasone

efficacy and toxicity of CCRT. This trial was designed to establish the optimal dose of DeVIC chemotherapy in combination with 50 Gy radiotherapy and then to historically compare the efficacy of this regimen with radiotherapy alone. This is the first prospective study for untreated, localized nasal NK/T-cell lymphomas that incorporated an adequate sample size and three central reviews (i.e., a central pathology review, a radiation quality assurance program, and a central CT review for efficacy assessments).

Patients with stage IE disease or contiguous stage IIE disease with cervical lymph node involvement were eligible for the trial. The protocol treatment comprised CCRT with relatively high-dose radiotherapy and chemotherapy with MDR-non-related agents and etoposide. Figure 6.1 depicts the treatment protocol for the trial (JCOG0211). In phase I, two dose levels of carboplatin, etoposide, and ifosfamide were evaluated. A two-thirds dose of DeVIC was then established as the recommended dose due to hematologic toxicity and infection.

In phase II of the trial, 27 patients who were treated with the recommended DeVIC dose (two-thirds) were evaluated. Compared to the historical control group [72], the median age was higher and B symptoms and cervical node involvement were more frequent in this study population. The median follow-up time was 32 months, with a range of 24–62 months. The primary endpoint, the 2-year OS, was 78% (90% CI, 61–88%; 95% CI, 57–89%), which was superior to the historical control of radiotherapy alone (45%) [72]. The 2-year progression-free survival (PFS) rate was 67%, the CR rate was 77%, and the overall response rate was 81%. The 2-year planning target volume control rate was 96%. Only one patient experienced loco-regional failure. Toxicity was mild and manageable in patients treated with the recommended dose, and no treatment-related deaths occurred. The most common grade 3 non-hematologic toxicity was mucositis related to radiation. From these data, it was concluded that CCRT using MDR non-related agents and etoposide is a safe and effective treatment for localized nasal NK/T-cell lymphoma, thus providing a basis for subsequent investigations.

A Prospective Trial of CCRT in a Korean ENKL Cohort

Subsequently to the aforementioned JCOG-LSG trial, a Korean group (Consortium for Improving Survival of Lymphoma; CISL) reported promising

results from a phase II study of CCRT followed by ICE (ifosfamide, carboplatin, etoposide)-like chemotherapy for localized nasal NK/T-cell lymphoma [54]. Weekly cisplatin (30 mg/m^2, four treatments in total) was used for CCRT as a radiation sensitizer. The median total dose of radiotherapy was 40 Gy, ranging from 40 to 52.8 Gy. After 3–5 weeks, three courses of cisplatin-containing chemotherapy, a VIPD chemotherapeutic regimen (etoposide 100 mg/m^2, days 1–3; ifosfamide 1,200 mg/m^2, days 1–3; mesna 240 mg/m^2, days 1–3; cisplatin 33 mg/m^2, days 1–3; and dexamethasone 40 mg/day, days 1–4) was initiated. The protocol treatment was designated CCRT-VIPD.

Thirty patients were enrolled in this study. The CR rate at the best response was 80%, [54, 86] which was superior to the historical control group used [70]. At the median follow-up of 24 months, the estimated 3-year OS and PFS rates were 86 and 80%, respectively [54, 86]. Toxicities during CCRT were reported to be mild. However, grade 3 or 4 infections occurred in 60% of the patients during the VIPD therapy, and two patients died of infection. The researchers concluded that localized nasal NK/T-cell lymphoma is best treated with frontline CCRT [54].

Current Optimal Treatment Strategy for Localized Nasal NK/T-Cell Lymphoma

Table 6.4 summarizes the results from the two prospective studies of CCRT for localized nasal NK/T-cell lymphoma. The JCOG0211 study was characterized by relatively high-dose radiotherapy supported by a quality assurance program, short treatment duration, and acceptable local toxicities. The Korean study showed promising results in terms of the estimated PFS. However, because it was initiated 3 years later than the JCOG0211 study, the excellent estimated PFS may have been obtained by strict baseline evaluation using new diagnostic and staging procedures, such as FDG-PET or in situ hybridization for EBER in bone marrow material [27]. No patients experienced disease progression during the CCRT with cisplatin.

It is of interest that the profile of toxicity was quite different between the two studies. The most severe and frequent non-hematologic toxicity reported for the RT-2/3DeVIC therapy was mucositis due to radiation. In the Korean study, two treatment-related deaths due to infection

Table 6.4 Comparison of the results from two prospective clinical trials of concurrent chemoradiotherapy for localized nasal NK/T-cell lymphoma

	JCOG0211 study [53]	Korean study [54, 86]
Study design	Phase I/II	(Pilot study→) Phase II
Registration period	Sep 2003–Dec 2006	Apr 2006–Oct 2007
Median follow-up period (months, range)	32 (24–62)	24 (17–37)
Number of patients in Phase II	27	30
Total dose of radiotherapy	50 Gy (supported by quality assurance program)	40 Gy (median)
Concurrent chemotherapy regimen	2/3 DeVIC	Cisplatin alone
Time to completion of the treatment	9 weeks	16–18 weeks
CR rate (%)	77	80[a]
OS rate (%)	78 (2-year)	86 (3-year;estimated)
PFS rate (%)	67 (2-year)	80 (3-year;estimated)
Planning target volume (local) control rate (%)	96 (2-year)	93 (3-year;estimated)
Most common toxicity	Local toxicities due to radiation	Infection (two treatment-related deaths)

[a]At the best response

were observed during VIPD chemotherapy. It is likely that infection during CCRT-VIPD therapy is also more severe and frequent compared with the JCOG 0211 study.

Although there are several problems in analyzing the endpoints of the Korean study [86], these two trials showed promising results for CCRT in patients with localized nasal NK/T-cell lymphoma, particularly in terms of the excellent local control. From these data, CCRT using MDR non-related agents and etoposide is recommended for the current standard of care for this disease, partly because it is the first-line treatment that seems to be superior to radiotherapy alone in prospective studies of localized nasal NK/T-cell lymphoma. If it takes considerable time (e.g., more than 1 month) to prepare for frontline radiotherapy, chemotherapy with non-MDR-dependent drugs with sandwiched radiotherapy deserves consideration [59], although there is no prospective data regarding the efficacy of such a treatment strategy.

High-Dose Chemotherapy with Autologous Hematopoietic Stem Cell Transplantation

The benefits of consolidative high-dose chemotherapy with autologous hematopoietic stem cell transplantation (HSCT) as a first-line treatment have yet to be established. In a retrospective, multicenter study in a Korean cohort of 262 patients, 16 individuals underwent high-dose chemotherapy with autologous HSCT [87]. Subgroup analyses did not reveal any benefit of autologous HSCT in patients with localized disease, Group 1 or 2 NK-PI, or an involvement of the upper aerodigestive tract [87].

A multinational, matched, controlled study from East Asia suggested that high-dose chemotherapy is beneficial for patients in CR with a high NK-PI at diagnosis [88]. This study was based on long-term follow-up data, but no consideration was given to the heterogeneity of treatment, such as the chemotherapeutic regimen or the timing of the radiotherapy.

Treatment of Systemic, Relapsed or Refractory Cases of ENKL

Chemotherapy

The treatment outcomes for stage IV, relapsed or refractory patients with ENKL using conventional chemotherapy are extremely poor. For example, in the case of CHOP(-like) chemotherapy, the overall response rate is 36% for newly diagnosed stage IV diseases but <10% in relapsed or refractory cases. Two promising results for non-anthracycline-containing regimens in the treatment of nasal NK/T-cell lymphoma have been reported. The first is a prospective study of newly diagnosed advanced-stage ENKL with nasal involvement in a patient group from Mexico. In this trial, the patients were given six courses of CMED chemotherapy (cyclophosphamide, methotrexate, etoposide, and dexamethasone), with a sandwiched radiotherapy of 55 Gy after three courses, in patients with facial involvement [89]. The second study in a Chinese cohort involved treatment with a salvage chemotherapeutic regimen (L-asparaginase, vincristine, and dexamethasone) after a first-line anthracycline-containing chemotherapy followed by involved-field radiotherapy (median, 56 Gy) in patients with relapsed or refractory nasal NK/T-cell lymphoma [90]. Although the CR rates in these two studies exceeded 55%, this high level of efficacy has not been validated in other studies. The high-dose radiotherapy that was used in both studies makes it difficult to evaluate the efficacy of the chemotherapeutic regimens themselves. Moreover, high-dose radiotherapy is not a treatment option for patients who have already received involved-field radiotherapy during their first-line therapy for localized nasal NK/T-cell lymphoma. Because it is known that there are long-term survivors among patients with advanced-stage, relapsed or refractory ENKL who have undergone HSCT, development of an effective chemotherapeutic regimen for these patients may be an important initial step in improving the treatment outcome.

In 2004, Japanese core members of NKTSG and other colleagues in East Asia formulated a

new chemotherapeutic regimen that comprises the steroid dexamethasone, methotrexate, ifosfamide, L-asparaginase, and etoposide (SMILE). The design of the SMILE regimen was based on several considerations. Etoposide was selected due to its in vitro and in vivo efficacy for NK-cell neoplasms and other EBV-associated diseases [81–83]. L-asparaginase is an anticancer drug that hydrolyzes serum asparagines and deprives lymphoid malignant cells of this required amino acid. This agent also induces the selective apoptosis of NK-cell lymphoma cells in vitro [91], and successful therapeutic results in NK-cell lymphoma have been reported using L-asparaginase, either alone or in combination with other drugs [90, 92, 93]. In a clinical trial for pediatric acute lymphoblastic leukemia, dexamethasone was reported to be more effective than prednisolone in ameliorating the adverse reactions to L-asparaginase [94]. Methotrexate and ifosfamide are unaffected by the MDR phenotype and are components of regimens that have been previously reported to be effective against ENKL. In the SMILE protocol, methotrexate was scheduled on day 1 to precede the other drugs because there is a possibility of antagonistic effects upon coadministration with etoposide and ifosfamide but synergic effects when preceding etoposide. The additional three drugs were scheduled for days 2–4 because the simultaneous use of etoposide and ifosfamide can lead to additive effects [95].

Given that advanced-stage ENKL and ANKL are extremely rare, a prospective therapeutic trial for these diseases is difficult to conduct. To overcome this problem, an international multicenter cooperative phase I study in East Asia was undertaken [95]. Patients with newly diagnosed stage IV, who had relapsed or refractory disease after first-line chemotherapy, and were between 15 and 69 years old with a PS of 0–2 were eligible. Six patients with ENKL were enrolled at Level 1 in the study. One treatment-related death due to infection occurred, but no other grade 4 nonhematologic toxicities were observed. After the first three patients were enrolled, a protocol revision stipulating the initiation of G-CSF from Day 6 was made. Major toxicities in six patients included grade 4 neutropenia and grade 3 hyponatremia. Three patients obtained a CR, with the remaining three cases showing PR, no response, and were not evaluable, respectively. The CR rate was 50% (3/6), and the overall response rate was 67% (4/6). The researchers concluded that a Level 1 dose with G-CSF support is recommended for further evaluation.

In the subsequent phase II study of SMILE, patients with ANKL were not eligible because none of these patients had been enrolled in phase I [95]. The primary endpoint was an overall response rate after two courses of SMILE chemotherapy thirty-nine patients were eventually enrolled and 38 patients were eligible. They had a median age of 47 years, and 20 of these patients had newly diagnosed stage IV disease. Because the first two patients died of a grade 5 infection, a protocol revision was made stipulating an awareness of infection and incorporating a lymphocyte count of 500/mm^3 or more into the eligibility criteria. There were no subsequent treatment-related deaths. The overall response rate, which was the primary endpoint of the trial, was 79% (95% CI, 65–89%), greatly superior to the historical control rate of 35%. The CR rate was 45%. The major toxicities were neutropenia, infection, and liver damage. These results indicate that SMILE chemotherapy is an effective induction therapy for newly diagnosed stage IV, relapsed or refractory ENKL [96]. Indeed, this regimen is now being used in clinical practice in East Asia [59]. However, careful monitoring for severe myelosuppression and infection is important when using this protocol.

Due to the high efficacy of this treatment against ENKL, a clinical trial incorporating a first-line use of SMILE chemotherapy for localized nasal NK/T-cell lymphoma may warrant investigation. However, because L-asparaginase can cause severe adverse drug reactions, such as thrombosis, hypofibrinogenemia, and pancreatitis, the efficacy and feasibility of first-line chemotherapeutic regimens containing this agent must be evaluated carefully.

Stem Cell Transplantation

To improve the therapeutic results in the treatment of ENKL, different HSCT settings have

been attempted for patients with ENKL. The first case series were reported by Liang and colleagues in 1997 [97]. Three relapsed localized nasal NK/T-cell lymphoma patients underwent high-dose chemotherapy with cyclophosphamide, carmustine, and etoposide, supported by autologous bone marrow transplantation. Two of the patients were reported to be disease-free more than 12 months after transplantation. Subsequent reports provided data for small numbers of patients who had undergone various kinds of transplantation, such as autologous HSCT, tandem autologous HSCT, allogeneic HSCT, and cord blood transplantation. Two independent Japanese surveys of HSCT in the treatment of ENKL further revealed that some long-term survivors do emerge among patients with advanced-stage or relapsed/refractory disease following allogeneic HSCT [98, 99]. Survival curves for patients who underwent allogeneic HSCT often show a plateau, suggesting that this treatment may be curative for a fraction of patients.

Development of Novel Biological and Targeted Therapies

Because NK-cell neoplasms are rare, there have been very few clinical trials of novel agents specifically targeting ENKL. NK-cell neoplasms have been included in clinical trials of various kinds of novel agents for peripheral T-cell lymphomas or chemotherapy-resistant B-cell lymphomas in western countries. In East Asia, bortezomib and alemtuzumab have been investigated in terms of their efficacy against ENKL. Bortezomib induces apoptosis in the tumor cells of NK-cell leukemia and lymphoma [100]. Further, one of three patients with ENKL achieved CR in a previous phase I study of CHOP chemotherapy with bortezomib for advanced T or NK-cell lymphoma [101]. However, because CHOP is not effective against ENKL, other chemotherapy regimens in combination with bortezomib may warrant further study. Alemtuzumab, a humanized anti-CD52 antibody, is available in some countries in East Asia, and experience with this agent in the treatment of ENKL has been accumulating. However, its efficacy seems to be temporal in most patients with relapsed or refractory ENKL. The relatively low incidence (25%) of CD52 expression in the tumor cells of ENKL [102] may also diminish the clinical usefulness of this agent in ENKL.

Some of the new anticancer agents that have been developed in western countries are anticipated to show efficacy against ENKL. For example, pralatrexate is a folate antagonist that achieves a greater intracellular accumulation than other anti-folate drugs such as methotrexate. A relatively high response rate in patients with peripheral T-cell lymphomas in a phase II-I-II trial for relapsed or refractory peripheral T-cell lymphomas has been reported for this drug [103]. No patients with ENKL were enrolled in this trial, but because of its similarity with methotrexate (a key agent in the treatment of ENKL), pralatrexate is expected to be effective against this disease. Another example of a potentially effective new drug against ENKL is the histone deacetylase inhibitor vorinostat. This agent shows significant induction activity in the EBV lytic cycle in EBV-positive carcinoma cell lines [104]. In combination with azacitidine, vorinostat is being investigated in a clinical trial for the treatment of ENKL and nasopharyngeal carcinoma. Finally, siplizumab, a humanized monoclonal antibody against CD2, may be a viable candidate as a targeting therapy for ENKL, because CD2 is expressed in most ENKL tumors. A phase I trial of siplizumab in 29 patients with T-cell malignancies revealed that this agent could decrease the expression of CD2 and deplete both T-cells and NK-cells. However, this trial had to be terminated because of a high incidence of EBV-positive lymphoproliferative disease (14%) [105]. In an attempt to overcome this issue, a clinical trial of a rituximab-containing chemotherapy with siplizumab is now being conducted in the United States.

Conclusions

NK-cell neoplasms are rare and aggressive diseases, but an increased understanding and recognition of distinct disease entities, the emergence of

better diagnostic procedures, recent multicenter prospective trials of new treatment strategies in East Asia, and the development of novel agents in western countries have all led to an improvement in the otherwise poor prognosis for these diseases. Further collaborative efforts on an international scale are needed to further improve the future treatments and outcomes for patients with NK-cell neoplasms.

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