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Emerging Insights on the Pathogenesis and Treatment of Extranodal NK/T Cell Lymphomas (ENKTL)

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Abstract

Extranodal NK/T-cell lymphoma (ENKTL) is a rare aggressive extranodal non-Hodgkin lymphoma (NHL) universally associated with Epstein-Barr virus (EBV). ENKTL most commonly occurs in non-elderly immune competent males in Asia and South America. A number of antecedent lymphoproliferative disorders (LPDs) have been described in Asian and South American patients, but the majority of Caucasian ENKTL patients have no known preceding LPD or underlying immunodeficiency. Other than EBV, no environmental or extrinsic factor has been implicated in oncogenesis. The precise mechanisms by which EBV infects NK or T cells and the virus' role in the pathogenesis of ENKTL have not been fully deciphered. However, a number of recent discoveries including disturbances in cell signaling and mutations in tumor suppressor genes have been identified, which are providing insights into the pathogenesis of ENKTL. In this

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review, we highlight the molecular, viral, and genetic underpinnings of ENKTL and discuss potential therapeutic implications.

Introduction

Early descriptions of destructive nasal tumors, consistent with extranodal NK/T-cell lymphoma (ENKTL), date back over 100 years (McBride, 1991; Woods, 1921), but the cell of origin in cases of “lethal midline granuloma” or “rhinitis gangrenosa progressiva” (other terms included polymorphic reticulosis or malignant midline reticulosis) was not identified until 1982 (Ishii *et al.*, 1982). Ishii and colleagues showed that the malignant cells within these lesions reacted with antibodies against T-cell antigens but not B-cell antigens. Subsequently, antibodies against the CD56 antigen questioned the T-cell lineage of the malignant cells (Kanavaros *et al.*, 1993; Suzumiya *et al.*, 1994; Wong *et al.*, 1992). Over the past 35 years, a body of evidence has emerged supporting the notion that the majority of these tumors are of natural killer (NK) cell origin, with germline T-cell receptor gene configurations (Ho *et al.*, 1990; Wong *et al.*, 1992). The explanation for the original finding describing this neoplasm as a T-cell lymphoma was due to the reactivity of polyclonal anti-CD3 antibodies against the cytoplasmic subunit (ε-chain) of the CD3 molecule, which is preserved in NK-cells (Chan *et al.*, 1996), in contrast to surface CD3, which is typically expressed in mature T-cells but not NK-cells. As a result of the initial misunderstanding, this NHL was designated as an NK/T-cell lymphoma and formally incorporated into the World Health Organization (WHO) classification of hematologic malignancies in 1999. It is further defined as *extranodal* as approximately 75% of cases present in the upper aerodigestive tract (UADT) with frequent angioinvasion and necrosis. *Extranasal* (non-UADT) ENKTL most commonly involves the gastrointestinal tract, skin, testis, lung, and soft tissue. The neoplastic cells are known to contain cytotoxic granule associated proteins [granzyme B, perforin, and T-cell restricted intracellular antigen (TIA-1)] and EBV-encoded RNAs (EBERs) (Chan *et al.*, 1994; Harabuchi *et al.*, 1990; Kawa-Ha *et al.*, 1989). Classically, the neoplastic cells are described as activated NK-cells with expression of CD2, CD56, cytoplasmic CD3ε+ (surface CD3-), and germline T-cell receptor (Swerdlow *et al.*, 2016). Several reports have documented the expression of killer-cell immunoglobulin-like receptors (KIRs) further validating their NK-cell phenotype (Dukers *et al.*, 2001). A small subset of cases demonstrates a cytotoxic T-cell phenotype (sCD3+, CD56- with α/β or less frequently γ/δ TCR expression) (Pongpruttipan *et al.*, 2012). In the recently revised WHO Classification, “ENKTL, nasal type” remains the only category for this neoplasm irrespective of cell of origin or site of disease.

Predisposing Factors in ENKTL

ENKTL is male predominant (~2:1) and more common in East Asia and Central/South America compared to North America and Europe. Within North America and Europe, ENKTL is more common among Asian Pacific Islander and non-white Hispanics. Thus, there appears to be a genetic predisposition for increased risk of ENKTL in these populations. In other EBV+ lymphoproliferative disorders (LPDs) higher rates of T/NK-cell infection occur in Asian cohorts (Cohen *et al.*, 2011b). It has also been shown that particular

human leukocyte antigen (HLA) subtypes affect the success of T-cell surveillance for EBV and thereby influence a recipient's predisposition to EBV-associated lymphomas (Reshef *et al.*, 2011). For example, Lustberg and colleagues showed that the HLA-B8 was associated with T-cell post-transplant lymphoproliferative disorder (PTLD) (Lustberg *et al.*, 2015). *In vitro* studies suggest that ENKTL cells retain major histocompatibility (MHC) class I and class II antigen processing function and can be recognized by CD8+ and CD4+ T cells targeting proteins produced by EBV [i.e., latent membrane protein-2 (LMP2) and Epstein-Barr nuclear antigen-1 (EBNA1)]. It remains unclear if specific HLA subtypes are more common in ENKTL. We hypothesize that there are certain HLA subtypes more common in Asian males that account for the higher incidence in this cohort. The genetic susceptibility to ENKTL remains an interesting area of discovery in ENKTL along with other EBV-associated T/NK-cell LPDs.

Asian and U.S. cancer registry data show significantly lower rates of ENKTL in U.S. Asians compared to native Asians (Bassig *et al.*, 2016). This suggests that in addition to increased genetic susceptibility in some races, there may be environmental factors more common in certain areas of the globe contributing to the increased incidence of ENKTL in the East. Co-factors such as chronic inflammation, co-infection(s), time of primary EBV infection, malaria (Rickinson, 2014), and HIV (Carbone *et al.*, 2009), which are implicated as cofactors in the lymphomagenesis of EBV-associated Burkitt lymphoma, have been suggested (Rickinson, 2014). In ENKTL, there are no co-factors, other than EBV, that are clearly associated; however, a subset of ENKTL cases is preceded by chronic inflammation or an LPD. Well characterized antecedent LPDs include chronic active EBV (CAEBV) (Jones *et al.*, 1988; Kawa-Ha *et al.*, 1989; Kimura *et al.*, 2001; Ohshima *et al.*, 1998), hemophagocytic lymphohistiocytosis (HLH), hypersensitivity to mosquito bite (HMB) (Ishihara *et al.*, 1997; Kawa *et al.*, 2001), and hydroa vacciniforme-like lymphoma (HVLL) (Quintanilla-Martinez *et al.*, 2013). Predominantly in patients from South America HVLL is reported to precede ENKTL (Quintanilla-Martinez *et al.*, 2013). HMB is shown to precede several NK-cell LPDs, including NK-cell CAEBV and ENKTL (Kawa *et al.*, 2001; Takahashi *et al.*, 2011). Unifying links between this spectrum of LPDs are EBV infection and inflammation. Previously studied factors related to EBV+ T/NK-cell growth include interleukin-9 as an autocrine growth factor (Nagato *et al.*, 2005; Yang *et al.*, 2004), interleukin-10 as an enhancer of responsiveness to interleukin-2 (Harabuchi *et al.*, 2009), the interferon gammainducible protein 10 as a promoter of tumor invasion (Moriai *et al.*, 2009), and CD70 as a cell surface receptor for soluble CD27, which is a paracrine growth factor (Yoshino *et al.*, 2013). This work underscores the importance of the microenvironment in ENKTL and dependence of a cross-talk with surrounding cell types for adequate EBV-infection of NK/T cells and subsequent growth and survival. This is exemplified by recent studies showing that monocytes, one of the major contributors to the nasal tumor infiltrate, deliver interleukin-15 to EBV-positive NK/T cells, inducing the EBV latent membrane protein-1 (LMP1) oncogene expression and secretion of the LMP1-inducible cytokine IP10 which works as a chemoattractant to drive monocyte recruitment (Ishii *et al.*, 2012). In summary there remain several well described antecedent LPDs; however, they do not consistently and reproducibly result in progression to ENKTL. Thus, additional genetic, environmental, or microenvironmental triggers are likely relevant to progression to ENKTL.

The majority of EBV+ lymphomas are of B-cell origin and often associated with a pre-existing primary or acquired immune deficiency. For example, post-transplant lymphoproliferative disorder (PTLD) can result from EBV driven lymphoproliferation and evolution to lymphoma in patients receiving iatrogenic immunosuppression. “EBV+ diffuse large B-cell lymphoma (DLBCL) of the elderly” (changed to “EBV+ DLBCL, NOS” in 2016 WHO Classification) with a median age of 77 years is thought to develop secondary to age related immune senescence (Castillo *et al.*, 2016). Whereas, the vast majority of ENKTL patients have no known underlying immune deficiency with a median age at diagnosis of 55 years. Recent reports show that EBV+ DLBCL can be seen in young individuals and may be associated with a permissive microenvironment with upregulation of programmed death ligand 1 (PD-L1) and increased indoleamine 2,3-dioxygenase (IDO) positive cell content (Beltran *et al.*, 2011; Nicolae *et al.*, 2015; Ok *et al.*, 2015). Similarly, a subset of ENKTL express PD-L1 (Kim *et al.*, 2016), which may be prognostic (Kim *et al.*, 2016). Takahashi *et al.* (2011) showed that younger ENKTL patients (i.e., <50 years of age) more frequently had B symptoms, worse performance status, advanced stage, and more sites of extranodal involvement. Future studies should focus on improving our understanding of the tumor microenvironment, immune response to EBV, and clinically unapparent immune dysregulation, which may differ by age or cohort. Ultimately, it is possible that specific genetic alterations or immune defects may predispose these clinically immune competent individuals to EBV-associated neoplasms.

NK/T-cells as a Target for EBV Infection

The first evidence that EBV infection was implicated in the progression to T or NK-cell neoplasms came from a report describing three patients (two of them adults) with clinical and serologic features of CAEBV who subsequently developed fatal T-cell lymphoma (Jones *et al.*, 1988). Since then, the incidence of EBV T/NK-cell infection in published studies has been conflicting. Recently, a Japanese study compared the cellular target of EBV infection in the peripheral blood of patients with EBV-associated HLH, CAEBV, and infectious mononucleosis (IM). EBV infection was predominantly in CD8+ T-cells in EBV-associated HLH, whereas the dominant EBV infected cell populations were CD4+ T-cells and CD16+ NK-cells in CAEBV. In IM patients the predominant infected cell type was B-cells (Kasahara *et al.*, 2001). In a study of a U.S. CAEBV patient cohort, EBV infected predominantly B-cells, whereby 58% (11/19) had a “B-cell disease” (Cohen *et al.*, 2011b). With the exception of CAEBV, it’s unknown how frequently EBV infects T or NK-cells in non-Asian patients. These studies highlight the differences in rates of T/NK-cell infection across races. The reason for this remains an active area of investigations but could be related to genetic (i.e., HLA) differences, immune response, or variation in the viral genome. Interestingly, U.S. patients with CAEBV showed reduced NK-cell numbers and progressive loss of B-cells with hypogammaglobinemia; whereas patients with CAEBV from Japan had normal or increased numbers of NK cells (Cohen *et al.*, 2011b), further highlighting the geodemographic differences in response to EBV infection. Irrespective of the differences in risk of T/NK-cell infection across races, it remains unclear how frequently EBV T/NK-cell infection progresses to T/NK-cell neoplasms.

Variations in the viral genome may predispose individuals to developing EBV-associated LPDs. EBV can be divided into two major types, mainly based on differences observed in EBNA genes (Dambaugh *et al.*, 1984; Rowe *et al.*, 1989). Recent work, including a large-scale sequencing study of EBV isolates from multiple tumor types and healthy carriers (Palser *et al.*, 2015), suggests that while the distinction between EBV type 1 (EBV-1) and type 2 (EBV-2) is accurate and reproducible, the genomic diversity of EBV is greater than previously recognized (Chang *et al.*, 2009). EBV-1 is more prevalent in the developed world (e.g., U.S., Europe, Asia) whereas EBV-2 is encountered more frequently in equatorial Africa. The impact that this diversity may have on the oncogenic properties of the virus remain unknown. EBV-1 readily transforms B-cells in culture, leading to the outgrowth of immortalized lymphoblastoid cell lines (LCL), while EBV-2 is poorly transforming (Dolan *et al.*, 2006; Rowe *et al.*, 1989). Recently, Coleman *et al.* (2015) showed that EBV-2 is able to efficiently infect CD8+ cytotoxic T-cells and induce proliferation and alter cytokine expression, although the relevance that this in vitro model may have on the development of ENKTL remains to be defined.

A recent French study in ENKTL patients detected EBV-1 in tumor and blood from all French natives (n=11), and EBV-2 in blood of two patients from Africa (corresponding tumor was not assessed). A control cohort without malignancy revealed only the presence of EBV-1 in blood (Halabi *et al.*, 2016), which agrees with previously published results (Chiang *et al.*, 1996; 1999; Kim *et al.*, 2003; Kuo *et al.*, 2004; Nagamine *et al.*, 2007; Suzumiya *et al.*, 1999). This study found a recurring 30 bp deletion (del30) in the LMP1 gene in 6 of 13 patients, which has been reported in 86–100% of Asian patients (Chiang *et al.*, 1996; Kim *et al.*, 2003; Kuo *et al.*, 2004; Nagamine *et al.*, 2007; Suzumiya *et al.*, 1999; Tai *et al.*, 2004). Other clonal variations were also reported, and in patients who achieved complete remission the wild-type form of LMP1 was more commonly detectable after treatment. Further investigation and comparison into clonal variations of EBV among U.S./European, Asian, and African cohorts at diagnosis and longitudinally after treatment is warranted. It's unclear if these differences have any impact on the spectrum of clinical presentation observed in Asian populations compared to North American, European, South American, or African cases. Highlighted in a recently published update on the epidemiology and clinical characteristics of ENKTL, the clinical spectrum of disease described in North America and Europe is narrower, compared to Asia. This variability could be secondary to the small sample size in the West, publication bias, and the differences across studies in defining "extranodal NK/T-cell lymphoma" versus other EBV-associated T/NK-cell LPDs. In summary, it remains to be defined if genetic susceptibility or immune response across races, or if differences in the viral genome account for the higher rates of T/NK-cell infection in Asia.

Role of EBV in ENKTL

While the incidence of ENKTL and the antecedent LPD varies across the globe, EBV-encoded transcripts and proteins are universally detected in the neoplastic cells of all ENKTL patients, irrespective of race (Au *et al.*, 2009; Chiang *et al.*, 1996; Minarovits *et al.*, 1994; van Gorp *et al.*, 1996). Additionally EBV genomes in tumor lesions are demonstrably clonal (Minarovits *et al.*, 1994; Tao *et al.*, 1995). EBV is a ubiquitous lymphotropic

gammaherpesvirus that infects >90% of the world population with a biological cycle of primary infection, latency, and lytic reactivation, whereby each latency type (I, II, or III) is characterized by a specific EBV transcriptional program, dictated in part by the level of immune competence of the host (Cohen *et al.*, 2011a; Young and Rickinson, 2004). The demonstration that EBV most efficiently infects and transforms resting B-cells *in vitro*, using CD21 and HLA class II as coreceptors (Fingeroth *et al.*, 1984; Li *et al.*, 1997; Nemerow *et al.*, 1985), and the observation that patients with IM have large numbers of circulating EBV-infected B-cells established the canonical view of EBV's distinct tropism for B-cells. Furthermore, these observations contributed to the development of valuable models of primary infection, replication, latency, and life-long persistence (Babcock *et al.*, 1998; Kurth *et al.*, 2000). According to these models, EBV initially infects and replicates in the oropharynx via co-receptors expressed on B-cells (HLA class II, CD21, and beta 1 integrin) and epithelial cells (beta 1, αvβ6/8 integrins) and then, under the selective pressure of an effective cell-mediated immune response, turns off most of its genes and enters a state of latency, with resting memory B-cells being the primary reservoir. In ENKTL the tumor most commonly occurs at sites of primary EBV infection. It is hypothesized that EBV infection in ENKTL occurs while NK-cells are attempting to kill an EBV infected cell target (Tabiasco *et al.*, 2003). The exact mechanism by which EBV infects T/NK-cells remains to be clarified.

The full spectrum of latent EBV genes expressed during the infection of B-cells includes six nuclear antigens [EBNA 1, 2, 3A, 3B, 3C, and leader protein (LP)], three latent membrane proteins (LMP1, 2A, and 2B), two small EBV-encoded RNA's (EBER1 and EBER2), and three clusters of micro-RNAs (miRs). Available evidence suggests that EBV genome copy numbers within ENKTL biopsies typically number no more than 20 virions per cell, consistent with latent episomal infection, although higher loads indicative of lytic virus replication have been observed and may have prognostic relevance (Hsieh *et al.*, 2007). EBV-encoded transcripts and proteins are detected usually with a latency program I, sometimes II (Asano *et al.*, 2013; Matsuo and Drexler, 2003; Minarovits *et al.*, 1994; Suzuki, 2014; Takahara *et al.*, 2006; Tao *et al.*, 1995; Xu *et al.*, 2001). It has been proposed that quantitation or monitoring of EBV genome copy number in serum or whole blood is a prognostic and predictive biomarker (Au *et al.*, 2004; Ito *et al.*, 2012; Jaccard *et al.*, 2011; Kanakry *et al.*, 2016; Suzuki, 2014; 2011; Wang *et al.*, 2012). However, since most quantitative PCR assays will detect both encapsulated virions and cell-free EBV DNA, viremia may simply reflect tumor shed DNA rather than virus replication. Therefore, the ideal prognostic and predictive EBV assay may vary by lymphoma subtype and the corresponding cell infected cell type. These observations require prospective evaluation alongside correlative virological studies, including in particular, a determination of the frequency and significance of EBV-harboring T and NK-cells in the peripheral blood at diagnosis and follow-up.

EBV is a highly adaptable tumor virus that can transform different cell types through constitutive activation of NF-kB, inhibition of apoptosis, activation of *MYC*, *BCL2*, and *NOTCH1*, and induction of extensive DNA methylation and genomic instability in the host cell. These effects are mediated by EBV latent proteins that function as transcriptional coactivators (EBNAs), signaling molecules (LMP-1, 2A), and epigenetic modifiers (EBNAs,

LMP-1), affecting a broad range of transcriptional programs and pathways in the host cell. Furthermore, the immediate early (IE) gene BZLF1, which activates EBV's lytic cycle, directly promotes B-cell lymphomagenesis and expression of BZLF1's activator XBP1 is associated with poor outcomes in B-cell lymphoma. EBV is also known to influence the T-bet/GATA3 axis (Th1/Th2) in T-cells, which is associated with survival in PTCL (Iqbal *et al.*, 2014). EBV causes upregulation of GATA3 expression *in vitro* (Siemer *et al.*, 2008), and the EBV-encoded miRBART20-5p can inhibit T-bet translation potentially blocking differentiation towards Th1 lineage (Lin *et al.*, 2013). The role of latent, lytic, and miRs in ENKTL are highlighted in Table 1.

Gene Expression Profiling and Genomic Studies

In addition to the rarity of ENKTL, biopsy specimens are typically small and necrotic, and the availability of unfixed tissue for molecular genetic studies is limited. Thus, tumor derived cell lines remain a valuable resource. However, given the clinical and heterogeneous overlap between T/NK-cell LPDs and ENKTL, cell line data might be misleading. Table 2 highlights several ENKTL cell lines, which we believe, based on the clinical context and cell of origin, may reflect ENKTL as defined in 2016. Several "NK//T-cell lymphoma" cell lines are more likely to be other T/NK-cell LPDs as currently defined in 2016, such as CAEBV (e.g., KAI3, SNK10, SNK16, SNT13, SNT15), ANKL (KHYG1, IMC-1), and other subtypes of leukemia (NKL, YT). While it has been shown that many of the "NK/T-cell lymphoma" cell lines share genomic alterations, the ENKTL cell lines disclose overexpression of a number of genes related to growth factor activity, apoptosis, cell growth, signal transduction and cell adhesion in comparison to CAEBV-derived cell lines (Zhang *et al.*, 2006). A model of pathogenesis in which the EBV oncoprotein LMP-1 induces the deregulation of p53, activation of C-MYC, and NFkB pathway, resulting in up-regulation of survivin has been proposed (Ng *et al.*, 2011).

It must be emphasized, that most studies have examined small cohorts of cases, sometimes heterogeneous, and that many were conducted in the late 1990s or early 2000s, a period when the diagnostic criteria for ENKTL, had not yet been fully established. Table 3 highlights several previous genetic, epigenetic, and miRNA studies in ENKTL. The most frequent chromosomal losses are observed at 1p, 6q, 11q, 13q, and 17p, and the gains most commonly at 1q, 2q, 7q, 17q, and 20q. The frequently observed DNA losses at chromosomes 6q and 13q suggested the implication of tumor suppressor genes mapping to these loci, in the pathogenesis of ENKTL. In more recent array comparative genomic hybridization (CGH) studies, the deletion of chromosome 6q (6q21–6q25) was found in 40–50% of ENKTL cases (Huang *et al.*, 2010; Iqbal *et al.*, 2009; 2011; Ko *et al.*, 2001; Nakashima *et al.*, 2005; Siu *et al.*, 1999; Sun *et al.*, 2003; Taborelli *et al.*, 2006).

Emerging Therapies

Anthracycline-based chemotherapy (i.e., CHOP) has now been shown to be largely ineffective, in part due to high levels of expression of P-glycoprotein (Wang *et al.*, 2008). Additionally, the SMILE (dexamethasone, methotrexate, ifosfamide, L-asparaginase, etoposide) regimen has demonstrated significant efficacy, and has become standard–

particularly in patients with advanced disease (Kwong *et al.*, 2012; Yamaguchi *et al.*, 2011). However, numerous novel therapies are promising in ENKTL. Brentuximab vedotin is an anti-CD30 antibody conjugate, which is efficacious in CD30-positive lymphomas (Younes *et al.*, 2010). CD30 is expressed in about 70% of ENKTL (Sabattini *et al.*, 2013). Lenalidomide is an immunomodulatory analog (IMiDs) with activity in lymphoid malignancies, including T-cell lymphomas, primarily through immune modulation (Kritharis *et al.*, 2015; Toumishey *et al.*, 2015). Further studies are required to examine the effectiveness of these agents in ENKTL. Additional immunomodulatory strategies include use of checkpoint inhibitors. As a single agent in an unselected cohort, checkpoint blockade was not very effective in T-cell neoplasms; however, EBV upregulates PD-L1 and in ENKTL checkpoint blockade has been more promising (Kwong *et al.*, 2017). Antigen-specific T-cells targeting immunodominant viral antigens from EBV have been used with dramatic success to treat EBV-associated PTLD (Doubrovina *et al.*, 2012; Heslop *et al.*, 2010). Patients with ENKTL are associated with type I/II EBV latency, where only weakly immunogenic EBV antigens LMP1, LMP2, and EBNA1 are expressed (Fox *et al.*, 2010). Despite this, of six ENKTL patients treated with LMP-CTLs, four had complete responses, which remained in remission at a median of 3.1 years after CTL infusion (Bollard *et al.*, 2014). This study indicates autologous T-cells directed to the LMP antigens can induce durable complete responses without significant toxicity. Development of EBV-CTLs against EBNA1 or novel methods of upregulating LMP1 may be better strategies in ENKTL. Unfortunately, EBV-CTLs require week(s) to produce and are thus often not available. A donor derived “off the shelf” bank of EBV-CTLs is being developed (Hanley *et al.*, 2012), and if the risk of graft versus host can be minimized, it will be an attractive option. Additional, targeted therapies include JAK inhibitors, and mTOR inhibitors. Ultimately, combinatorial or sequential strategies that optimally exploit genomic, viral, and immunologic properties of the tumor will succeed.

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Table 1

Viral Gene Expression in ENKTL.

Gene(s)	Primary Function	Major Findings (as it relates to gene expression in ENKTL)	Reference(s)
EBER	In B cells, some evidence indicates that EBER expression protects against apoptosis and contributes to proliferation.	Thought to function via TLR3 to amplify the inflammatory response in HLH, CAEBV, and IM; unknown in ENKTL.	Iwakiri et al. J Exp Med, 2009.
EBNA1	Ensure faithful transmission of the circularized EBV episome to daughter cells by facilitating its replication during cell division.	Partial silencing in EBV+ NK cell line reduced cell proliferation.	Ian et al. Cancer Biol Ther, 2008.
EBNA2	DNA binding protein, interacts with cellular RBPJk.	Absent by IHC in tumors.	Chiang et al. Int J Cancer, 1996.
EBNA3	Affect transcription of viral and cellular genes.	Absent.	Chiang et al. Int J Cancer, 1996.
LMP1	Classic oncogene in B-cell transformation; modulator of cell signaling; induces a number of antiapoptotic proteins includes BCL2; functions to constitutively activate the TNF receptor and functionally resembles CD40, providing growth and differentiation signals to B-cells.	Expression is seen in the vast majority of cell lines but this does not mirror in vivo situation. Microenvironmental factors and cytokines (e.g., IL2, IL10) may be influential in expression in tumors. By IHC, some ENKTL tumors are LMP1-.	Chiang et al. Int J Cancer, 1996.
LMP2	Facilitate immortalization and lytic cycle but are not essential for B-cell transformation; may drive proliferation and survival of B-cells in the absence of BCR signaling.	Expression typically absent by IHC, although LMP2 specific CD8+ T-cells recognize and kill cell lines and induce clinical responses in patients. Subsequently, a novel LMP2 transcript was identified, which may serve as the target.	Fox et al. Blood, 2010. Chiang. Int J Cancer, 1996.
BZLF	Immediate-early genes; expressed following lytic activation.	Negative ZEBRA IHC in tumors.	Chiang et al. Int J Cancer, 1996.
BHRF1 miRNA cluster		No expression in cell lines. Detected in rare cells suggesting that lytic transcripts are most likely expressed by rare cells entering lytic cycle.	Chiang et al. Int J Cancer, 1996.
BART miRNA cluster		BART miRNAs are increased in cell lines. Mir-BART9 seems to influence expression of LMP1 and cell growth. mir-BART20-5p inhibits translation of T-bet in EBV-infected YT lymphoma cells of NK-cell origin.	Ramakrishnan et al. PLoS One, 2011. Lin et al. Am J Pathol, 2013.

Table 2

Summary of ENKTL Cell Lines.

Cell Line	Patient Source	Cell Lineage
NKYS	19 year old female from Japan	NK-cell, εCD3+, CD56+
SNK6	62 year old, nasal tumor from Japan	NK-cell, εCD3+, CD56+
NK92	50 year old non-nasal tumor from Caucasian	NK-cell, εCD3+, CD56+
SNK1	24 year old from Japan	NK-cell, εCD3+, CD56+
SNT8	48 year old, nasal tumor from Japan	γδT-cell, CD3+, CD56+
NK-S1	Xenograft of nasal tumor from China (Loong et al. Leuk & Lymphoma, 2008)	εCD3+, TIA-1+, CD56-

Table 3

Summary of Genetic, Epigenetic, and miRNA Studies.

Authors	Methodology	Source	Findings
Yamaguchi et al. Cancer, 1995.	IHC and reverse transcription (RT) PCR	Ten Japanese patients with nasal NK/T-cell lymphoma	Nine of the 10 patients were P-glycoprotein positive by IHC. MDR1 mRNA was detected in all seven pts examined by RT-PCR.
Wong et al. BJH, 1997.	Conventional karyotype	7 CD56+ leukemia/lymphoma (2 extranasal, 1 nasal, 3 ANKL, 1 blastoid leukemia/lymphoma)	Del(6)(q21q25) is a recurring abnormality.
Siu et al. Am J Pathol, 1999.	CGH	Primary tumors from four nasal NK cell lymphomas, one nasal-type NK cell lymphoma, and five NK cell lymphomas/leukemias	Deletions at 6q16-q27 (four cases), 13q14-q34 (three cases), 11q22-q25 (two cases), and 17p13 (two cases).
Ko et al. Cytometry, 2001.	CGH	7 nasal ENKTL (6 NK-ENKTL, 1 T-ENKTL)	Frequent DNA losses at 1p, 17p, and 12q and gains at 2q, 13q, and 10q. Infrequent loss of 6q comprise several candidate tumor suppressor genes (PRDM1, HACE1, FOX03, AIM1, ATG5).
Nakashima et al. Genes Chromosomes Cancer, 2005.	Homemade array-based CGH	10 aggressive NK-cell leukemia cases and 17 ENKTL, nasal type	Gain of 1q23.1–24.2 and 1q31.3–q44), and loss of 7p15.1–p22.3 and 17p13.1 occurred significantly more frequently in ENKTL. Gain of 2q, and loss of 6q16.1–q27, 11q22.3–q23.3, 5p14.1–p14.3, 5q34–q35.3, 1p36.23–p36.33, 2p16.1–p16.3, 4q12, and 4q31.3–q32.1 were nonsignificantly more common in ENKTL.
Iqbal et al. Leukemia, 2009.	CGH & GEP	NK cell lines & 7 patients with NK-cell malignancies	PRDM1 was the most likely tumor promoting gene in del6q21. ATG5 and AIM1 may also participate in the tumor development and progression.
Huang et al. Blood, 2010.	GEP	9 tumors (1 T-cell ENKTL, 8 NK-cell ENKTL) & 2 cell lines (SNK6 and SNK8)	Compared to normal NK cells, tumors were closer to activated than resting cells and overexpressed several genes related to vascular biology, EBV induced genes, and PDGFRA. Integrative analysis also evidenced deregulation of the tumor suppressor HACE1 in the frequently deleted 6q21 region.
Jiang et al. Nature Genetics, 2015.	Whole exome sequencing	25 de novo ENKTL	Recurrent mutations were most frequently located in the RNA helicase gene DDX3X (20%), tumor suppressors (TP53 & MGA), JAK-STAT pathway molecules (STAT3 & STAT5B), and epigenetic modifiers (MLL2, ARID1A, EP300, & ASXL3).
Kucuk et al. Clin Cancer Res, 2015.	Global promotor methylation analysis	12 ENKTL and 7 NK cell lines (NK92, KHYG1, YT, SNK1, SNK6, NKYS, & KAI3)	Identified 95 genes with strong evidence for being silenced because of promotor methylation, including BCL2L11 (BIM), DAPK1, PTPN6 (SHP1), TET2, SOCS6, and ASNS.