Anaplastic Large Cell Lymphoma: After Twenty Years the Controversy Continues
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Introduction and Historical Perspective:

While evaluating a new antibody Ki-1 directed against an epitope on Reed-Sternberg cells, Harald Stein, Karl Lennert and others in Kiel, West Germany recognized a unique lymphoma composed of pleomorphic large cells with strong expression of the antigen detected by the Ki-1 antibody (now designated as CD30). This “Ki-1 lymphoma” had a prominent sinus growth pattern and was previously misdiagnosed as metastatic carcinoma, melanoma, malignant histiocytosis, or lymphocyte depleted Hodgkin lymphoma (HL). Important events in the definition of anaplastic large cell lymphoma (ALCL) in late 1980’s and early 1990s included inclusion in the updated Kiel2 and REAL3 classifications, the discovery by Delsol et al. that ALCL frequently expressed epithelial membrane antigen (EMA)4 and recognition by several groups of its association with a unique chromosomal translocation t(2;5)(p23;q35).5,7

Despite these advances, considerable debate continued concerning whether ALCL was an entity or a heterogeneous group of diseases based on variability in morphology (large cell with monomorphic and pleomorphic histology, small cell predominant, lymphohistiocytic, and cases that resembled HL, so called Hodgkin-related), immunophenotype (T-cell, null, and B-cell types) and clinical presentation (systemic with nodal and/or extranodal sites of disease, primary cutaneous, ALCL arising in association with other lymphoproliferative processes such as lymphomatoid papulosis, mycosis fungoides or HL), or in immunocompromised patients.

The ALK (Anaplastic Lymphoma Kinase) Gene and its Role in Classification of ALCL:

In 1994, Morris et al. cloned the t(2;5)(p23;q35) and identified a unique fusion gene formed from the N-terminal portion of the constitutively expressed nucleophosmin (NPM) gene involved in ribosomal shuttling between the nucleolus and the cytoplasm and the intracellular portion of a new receptor tyrosine kinase gene they called anaplastic lymphoma kinase (ALK).8 ALK is expressed in portions of the nervous system but is not normally present in lymphoid tissue. The fusion gene encodes a protein with a dimerization domain (contributed by NPM) that activates signaling through the ALK receptor tyrosine kinase. Other fusion proteins have been identified that create a similar dimerization domain but have only cytoplasmic ALK expression and lack the nuclear pattern seen with staining NPM-ALK as it dimerizes with normal NPM and is transported into the nucleus.9 ALK signaling leads to activation of multiple pathways, leading to enhanced proliferation, and inhibition of apoptosis.9

Antibodies created against the ALK protein10,11 lead to wide scale testing of ALCL cases and further definition of the spectrum of diseases included in ALCL.12-15 The ALK + subset of ALCL represents approximately 60% - 80% of ALCL overall and defines a distinct lymphoma predominantly occurring as a systemic disease in children (>90% ALK+) and young adults with a morphologic spectrum from a small cell predominant variant to typical large cell histology, and a T-cell or null cell phenotype with frequent expression of EMA and cytotoxic granule protein TIA-1. The ALK- group is heterogeneous and includes systemic ALCL usually in older adults,
primary cutaneous ALCL (PC-ALCL), and ALCL arising in other lymphoproliferative disorders or in immunocompromised patients.

**Pitfalls in the Diagnosis of ALK+ ALCL (Case presentation of the small cell variant of ALCL):**

The diagnosis of ALK+ ALCL has become relatively straightforward. The case submitted for the conference workshop is the index case of the small cell variant (SCV) of ALCL\(^\text{16}\) and is presented to illustrate potential pitfalls that remain even for ALK+ lymphoma.

The clinical history is that of a 17 year-old male with a several weeks of fever, weight loss, generalized adenopathy, and hepatosplenomegaly with elevated liver function tests. The clinical impression was viral hepatitis. The WBC was 45,000/mm\(^3\) with 58% lymphocytes. The initial specimens obtained for diagnosis were small biopsies from a skin rash and the liver. A limited work up showed a cutaneous perivascular and hepatic periportal T-cell infiltrate composed of small lymphocytes. A minor population of large CD30+ lymphocytes was present. A diagnosis of abnormal T-cell infiltrate compatible with viral infection was made. The illness progressed and a lymph node biopsy was obtained and showed a paracortical and sinus infiltrate with a predominance of small lymphocytes with irregular nuclei and pale to clear cytoplasm and evidence of hemophagocytosis in the sinuses. Immunostaining for CD30 revealed a minor population of predominantly large CD30+ lymphocytes with a prominent distribution around blood vessels. Immunophenotyping studies revealed an abnormal T-cell population with expression of CD2, CD7, CD8, CD25, CD30, HLA-DR, and EMA and loss of CD3 and CD5. Cytogenetic studies demonstrated a t(2;5)(p23;q35) in the tumor cells and a subset had a +X.

This case illustrates the features that make the diagnosis of the SCV difficult: young patient age (reported in some infants less than 6 months), clinical features suggesting a viral illness (fever, rash, hemophagocytic syndrome), partial effacement of lymph node architecture, predominance or small lymphocytes, and initial biopsies from extranodal sites. As CD30 is an activation antigen and can be expressed in numerous reactive conditions, other lymphoid proliferations due to viral infection, drug reactions, and lymphocyte recovery after stem cell transplant have to be excluded. CD30 is also expressed in tumor cells (usually a minor population) in other T-cell and even B-cell lymphomas, germ cell tumors, and other solid tumors. A particularly important feature in the diagnosis of the SCV is the predilection for the large, CD30+ cells to be distributed around blood vessels rather than randomly as would be seen in reactive processes or other lymphomas with CD30+ cells. The SCV is often a disseminated disease with involvement of lymph nodes and extranodal sites including the skin, liver, pleural fluid, bone marrow, peripheral blood.\(^\text{16, 17}\) Bone marrow examination for staging of ALCL should always include immunostaining for CD30 and ALK, if the tumor is ALK+. Overall, bone marrow involvement is present in at least 50% of cases of the SCV and much less common in other histologic types of ALCL (approximately 20% - 25%). When considering the SCV, peripheral blood films should be examined for the presence of large lymphocytes with finely vacuolated basophilic cytoplasm that, despite an elevated white blood count, may represent less than 1% of the white blood cells. In the case presented, rare large tumor cells and more numerous small lymphocytes with irregular nuclei were present in the peripheral blood and bone marrow. A large clonally rearranged band (representing at least 50% of the cells present) on a T-cell receptor beta chain rearrangement performed by Southern blotting on DNA isolated from the peripheral blood and the finding of a t(2;5) in 23/25 cells in the bone marrow (where large tumor cells were rare)
provided indirect evidence that the small cells were part of the neoplastic clone. Later studies confirmed the presence of the t(2;5) in the small lymphocytes by immunohistochemistry. This case also illustrates that although most cases of ALC (including the SCV) arise from CD4+ T-cells, a small subset CD8+. The SCV can transform to typical ALC morphology (more often monomorphic) and both histologies may be seen in a single lymph node. While ALK expression has been reported to be favorable, the SCV is aggressive. Recent evidence suggests decreased SHP1 phosphatase activation may be related to the aggressive nature of SCV.

Expression of myeloid antigens (CD13, and rarely CD117) and keratin (particularly KL1) can be seen in ALK + ALC and cause difficulty in the diagnosis, particularly if the tumor is of null immunophenotype. It should be also be pointed out that ALK expression alone is not diagnostic of ALC. Other ALK+ tumors include inflammatory myofibroblastic tumor and other soft tissue tumors, tumors of neural origin (neuroblastoma, glioblastoma) and a very rare ALK+ B-cell tumor (that lacks CD30 but expresses EMA and some CD4 and CD57).

**Primary Cutaneous ALC, the Most Well-defined Entity of the ALK- ALC Group:**

The CD30+ clonal lymphoproliferative disorders lymphomatoid papulosis (LyP) and primary cutaneous ALC (PC-ALC) represent approximately 30% of primary cutaneous lymphoid neoplasms and form a histologic and biologic spectrum. PC ALC arises in older patients (median 40 - 67 years) but can rarely occur in children. Lesions are typically solitary, or less frequently multiple, nodules or tumors (1 - 2 cm or greater) that are often ulcerated and occur on the extremities>head and neck>trunk. Tumor cells are present in large clusters and sheets with the exception of those neutrophil-rich or pyogenic variants. Pseudoepitheliomatous hyperplasia can lead to confusion with squamous cell carcinoma. Greater than 90% PC ALC are CD4+ T-cell proliferations with variable loss of pan-T-cell antigens. Approximately 75% express one cytotoxic granule protein (TIA-1 or granzyme B) and approximately 20% - 30% express EMA. CD56 expression has been reported in 12% - 75% and does not appear associated with a worse prognosis. Clusterin is expressed in the majority of cases.

Skin involvement is present in approximately 15% - 25% of systemic ALC. The importance of distinguishing PC ALC from secondary skin involvement in systemic disease is illustrated by the excellent prognosis of PC ALC with a disease related 5-year survival of > 90% as compared to the latter with a 5 year cumulative survival of 24% - 44%. Unfortunately there are no markers that reliably distinguish primary cutaneous and systemic ALC with secondary skin lesions, and careful staging and follow-up are indicated as 10% - 20% of patients may develop systemic disease (particularly those with multifocal lesions). The differential diagnosis of PC ALC includes transformed mycosis fungoides (MF) and is principally distinguished by the presence of small cerebriform lymphocytes and a previous history of patches and plaques in MF. Acute myeloid leukemia can rarely express CD30 and is distinguished by the presence of myeloid antigens (CD13, CD15, CD33, CD68, and myeloperoxidase), CD34, and the weaker more diffuse cytoplasmic expression of CD30.

LyP typically occurs in adults with a median age of 45 years and can rarely be present in children. Multiple papular, papulonecrotic, or nodular lesions (usually < 1 cm) that ulcerate and heal with a scar in 3 – 12 weeks are present. LyP may be chronic and recur for months or years. Other lymphomas (ALCL, MF, or HL) can develop concurrently, before, or after LyP in
5% - 20% of patients. ALCL and MF arising in a patient with LyP do not appear to have a more aggressive course.27,42 LyP is classified as Type A when scattered single or small clusters of dysplastic (often Reed-Sternberg-like) CD30+ large cells are present in a wedge-shaped perivascular infiltrate often admixed with acute inflammatory cells. LyP type B is the least common (<10%) variant and is band-like with epidermotropism and more cerebriform nuclei; CD30+ large cells are absent or sparse. LyP type C shows overlap with ALCL, but typically has little involvement of the subcutis; a history or regression is the most important distinguishing feature. The immunophenotype of LyP is similar to PC ALCL with a CD4+ (rarely CD8+) phenotype with EMA present in 31%.43 TIA-1 and/or granzyme B is expressed in 74% - 100%.44,45 CD56 has been reported in 0% - 50%.40,46 LyP must be distinguished from other reactive conditions with CD30+ large cells such as drug reactions (anti-convulsants, histamine receptor antagonists, anti-depressants, phenothiazines, calcium channel blockers, angiotensin converting enzyme inhibitors, and antibiotics) arthropod assault, viral infection (herpes virus and parapoxvirus), insect and spider bites, and lymphocyte recovery after marrow ablative therapy.

**Diagnosis and Classification of ALK- ALCL:**

Approximately 20% - 60% of ALCL are ALK- with more cases seen in older adults. Due to lack of a well-defined pathogenetic mechanism or specific clinico-pathologic features (other than PC-ALCL), hematopathologists have debated whether ALK- ALCL is a specific entity or a heterogeneous condition that should be included in peripheral T-cell lymphoma, unspecified (PTCL-U). Recent studies have shown common features shared by ALK+ and ALK- ALCL that are not typically present in PTCL-U. Defects in T-cell receptor signaling have been noted and include the lack of expression of the T-cell receptor alpha-beta protein (seen in > 90% of ALK+ and ALK- ALCL) as compared to approximately 10% of PTCL-U.47 Ninety-six percent of ALK+ and 40% of ALK- ALCL also lack expression of CD3 as compared to 29% of PTCL-U. ZAP-70, a tyrosine kinase that integrates cognate and co-stimulatory signals for downstream TCR signaling, is expressed in only 25% - 30% of ALCL (8% - 25% of ALK+ and 20% - 41% of ALK-) versus 59% - 74% of PTCL-U and 29% - 57% of PC ALCL.48,49 In addition, clusterin, a ubiquitous glycoprotein, is expressed in 82% - 100% of systemic ALK+ and ALK- ALCL and 41% - 100% of PC ALCL while only 3.5% of PTCL-U express clusterin.55,50,51 Although ALK+ and ALK- ALCL show differential gene expression on microarray analysis, both groups highly express kinase genes *LCK*, *protein kinase C*, *vav2* and *NKIAMRE* and anti-apoptotic molecules.52 Comparative genomic hybridization studies have also shown significant losses of 5q21 and 9p21-pter in PTCL-U that were not detected in ALK- ALCL.53

To make a diagnosis of ALK- ALCL there must be strict adherence to the characteristic cytology (large cell predominant population with abundant cytoplasm and pleomorphic, embryoid or hallmark or wreath-like nuclei) and strong expression of membrane and Golgi (dot-like cytoplasmic) CD30 expression in virtually every cell. In lymph nodes, some involvement of nodal sinuses should be seen. In ALK- ALCL with a null phenotype and genotype, care must be taken to exclude other tumors that may express CD30 (HL, carcinoma, and acute leukemia).

**Summary**

Since the original description of ALCL in 1985, there have been many difficulties and much controversy in its diagnosis and classification. Frequent involvement of extranodal sites, lack of specificity of CD30, expression of EMA and frequent lack of CD45RB (leukocyte common
antigen), and rare expression of myeloid antigens or keratin have contributed to the potential for misdiagnosis. Recognition of the (2;5)(p23;q35) translocation and abnormal expression of ALK in lymphoid tissue, as detected by commercially available antibodies, has led to clarification in the classification and diagnosis of ALCL and recognition of an ALK+ ALCL that occurs predominantly as systemic disease in children and younger adults, has a morphologic spectrum from large to small cell predominant, T or null cell phenotype, and frequent expression of EMA. ALK expression is usually associated with systemic disease and a better prognosis, but there are rare cases of ALK+ systemic ALCL and most cases of the SCV (which is typically ALK+) that have an aggressive course. Presently, the classification of ALK- ALCL (other than PC ALCL) remains controversial. Recent genetic and biologic discoveries suggest that ALK- ALCL is more closely related to ALK+ ALCL than PTCL-U.

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