ALK-positive anaplastic large cell lymphoma: an evolving story

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1. ABSTRACT

The current classification of lymphoid neoplasms is based on the integrated utilisation of morphological, immunohistochemical, genetic and clinical criteria to define disease entities. Anaplastic large cell lymphoma is a paradigm for the identification of a disease entity based on morphological observations and immunophenotype, which paved the way for the subsequent discovery of the characteristic cytogenetic abnormality the translocation t(2;5) (p23;q35). In 1994, the t(2;5) was cloned and the NPM-ALK fusion gene generated by this rearrangement was identified. The year 2014 marked the 20th anniversary of this seminal publication by Steve Morris et al. The discovery of anaplastic lymphoma kinase (ALK) has allowed the definition of a distinct entity within the clinically and pathologically heterogeneous group of CD30+ lymphomas. The diagnosis of ALK-positive ALCL has become straightforward due to the generation of the reliable monoclonal antibody ALK-1 that also has led to the recognition of the histologic spectrum of the disease. ALK-positive ALCL has evolved in the last 20 years to an exciting model for signal transduction studies and targeted therapy.

2. INTRODUCTION

Anaplastic large cell lymphoma (ALCL) represents a distinct type of non-Hodgkin lymphoma (NHL) of T or null phenotype with unique morphologic features and CD30 antigen expression (1). According to the 2008 WHO classification of Tumours of haematopoietic and lymphoid tissues (1) there are 2 forms of systemic ALCL based on the presence or absence of the characteristic cytogenetic abnormality, the t(2;5) chromosomal translocation, which juxtaposes the anaplastic lymphoma kinase (ALK) gene at 2p23 to the nucleophosmin (NPM) gene at 5q35, resulting in the expression of the chimeric protein called NPM-ALK (2). There are several variant translocations involving 2p23, all of which generate ALK fusion proteins capable of autodimerization, leading to constitutive activation of the ALK-tyrosine kinase, believed to initiate the process of lymphomagenesis. In contrast, ALK-negative ALCL was included in the 2008 WHO classification as a provisional entity, and is defined as a CD30+ T-cell neoplasm that is not reproducibly distinguishable on morphological grounds from ALK-positive ALCL, but lacks ALK gene rearrangement and expression, with most cases expressing T-cell-associated markers and cytotoxic granule-associated proteins (3). Interestingly, gene expression profiling (GEP) has shown that ALK-positive and ALK-negative ALCL have distinct signatures but show, nevertheless, similarities in their molecular profiles suggesting shared pathogenic mechanisms (4-7). In this review ALK-negative ALCL will be briefly considered in order to provide the framework for understanding the concept of ALCL.

3. HISTORICAL BACKGROUND

The history of what we know now as ALCL began in 1982 when the monoclonal antibody Ki-1 was raised against Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma (figure 1) (8). In 1985,
Stein et al. (9) identified a unique group of large cell lymphomas with anaplastic morphology suggestive of "malignant histiocytosis" exhibiting prominent sinusoidal invasion and uniform, strong expression of the antigen Ki-1. Subsequently, Ki-1 was identified as an activation antigen, designated CD30, and member of the tumor necrosis factor receptor family. Relatively soon it was recognized that CD30+ anaplastic large cell lymphoma represented a distinct clinicopathological entity and was incorporated in the revised Kiel classification in 1988 (10, 11) and in the revised European-American lymphoma classification in 1994 (12). The pathogenesis of ALCL began to deciphered in 1989 when three independent groups reported the t(2;5)(p23;q35) as a recurrent chromosomal abnormality in ALCL, a rare cytogenetic abnormality thought initially to be characteristic of malignant histiocytosis (13-15). The translocation was cloned by Morris et al. in 1994 (2), and was found to involve a novel receptor tyrosine kinase called anaplastic lymphoma kinase (ALK) on 2p23 and nucleophosmine (NPM) on 5q35. Therefore, last year we celebrated the 20th anniversary of the discovery of ALK. Many groups have since described additional translocations in which ALK is fused to other partners (16). Because ALK is not normally expressed in lymphoid tissue, the development of a specific monoclonal antibody against ALK has proven extremely useful for clinical studies and routine diagnosis (17), as it was demonstrated that the ALK-1 antibody also recognizes the different variant translocations. Therefore, immunohistochemistry has become the gold standard for the diagnosis of ALK-positive ALCL based on its sensitivity and specificity. Although initially recognized by traditional histology and immunophenotypic studies, ALK-positive ALCL has evolved to a distinct molecular entity and a model for signal transduction studies (16, 18-20) and targeted therapy (21, 22).

### 4. HISTOLOGIC FEATURES

According to the WHO classification (23) ALK-positive ALCL is a T-cell lymphoma consisting of lymphoid cells that are usually large with abundant cytoplasm and pleomorphic, often horseshoe- or kidney-shaped nuclei with an eosinophilic region near the nucleus (figure 2A-D). These cells have been referred to as hallmark cells because they are present in all morphological variants (24). Five morphological patterns can be recognized.

The most frequent variant is the "common pattern" which accounts for 60% of all the cases. In this variant the tumor cells have abundant cytoplasm that may appear clear, basophilic or eosinophilic with a prominent Golgi zone. The nucleus is often lobulated and may resemble Reed-Sternberg cells. The nuclear chromatin is finely dispersed or clumped with multiple small, basophilic nucleoli. In this variant hallmark cells are frequently seen. A striking feature of ALCL is its tendency to grow within the sinuses in a cohesive manner mimicking melanoma or carcinoma metastasis, although complete architectural effacement can also be seen. Another helpful feature is the perivascular distribution of the malignant cells forming rosettes around the blood vessels (24). An inflammatory background is invariably present but its intensity varies in the different morphological patterns of ALCL.

The small cell and lymphohistiocytic variants are next in frequency after the "common pattern" and are particularly important because they may be misdiagnosed as either atypical inflammatory lesions or peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS). (figure 3A-B) They seem to be related because tumors may contain a mixture of these two patterns or may appear alone or in combination in sequential biopsies. The presence of hallmark cells supports the diagnosis of
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The small cell pattern (5-10%) shows a predominant population of small cells with irregular nuclei and abundant clear cytoplasm (figure 3A) (25). Hallmark cells may be so abundant as to mask a small population of tumor cells in the background. The tumor cells tend to be smaller than in the "common pattern". Plasma cells are present; however, neutrophils and eosinophils are sparse or absent. As in the small cell pattern, the identification of hallmark cells mainly surrounding the blood vessels is a clue to the correct diagnosis.

The lymphohistiocytic variant (10%) is characterized by tumor cells admixed with abundant reactive pale histiocytes (figure 3B) (26). The histiocytes may be so abundant as to mask a small population of tumor cells in the background. The tumor cells tend to be smaller than in the "common pattern". Plasma cells are present; however, neutrophils and eosinophils are sparse or absent. As in the small cell pattern, the identification of hallmark cells mainly surrounding the blood vessels is a clue to the correct diagnosis.

The "Hodgkin-like pattern" (3%) is characterized by morphological features mimicking nodular sclerosis classical Hodgkin lymphoma (figure 3D)(27). These cases are usually resolved with immunohistochemical analysis. Other less frequently encountered patterns include the monomorphic variant, cases rich in multinucleated giant cells or cases with sarcomatoid features. The importance of recognizing these rare variants lies in the potential of misdiagnosis with serious clinical consequences. The use of immunohistochemistry for CD30 and ALK-1 helps to avoid misdiagnoses and allows a precise definition of ALK-positive ALCL.

5. CLINICAL FEATURES

ALK-positive ALCL is most common in children and young adults but can occur in older adults; however, most cases of ALCL in the elderly are ALK-negative (28). There is a slight male predominance (around 60%) in both ALK-negative and ALK-positive ALCL. ALCL represents approximately 20-30% of pediatric/adolescent NHL and 2-8% of adult NHL (29). In the pediatric age group fewer than 10% belong to the group of ALK-negative ALCL (30). The median age at presentation in the pediatric group is around 10 years with a range from 10 months to 17 years; however, ALCL rarely occurs in infants. Most patients with ALCL have advanced stage disease (ALK+, 65%; ALK-, 58%) and B-symptoms (54% - 75%) at presentation, especially high fever (28-30). ALCL usually presents with lymph node enlargement (approximately 90%) including mediastinal involvement in 36% of the cases. Extranodal disease is frequently found (40% - 68%) with skin, bone, and soft tissue being the most common sites. Soft tissue masses may sometimes be mistaken for soft tissue sarcomas. Patients with ALK-positive ALCL are more likely to have bone marrow or subcutaneous tissue involvement (28, 30). The incidence of bone marrow involvement is approximately 10% when analyzed by hematoxylin and eosin but the detection rate increases to approximately 30% with immunohistochemical analysis using CD30 or ALK1 antibodies (31). In contrast, patients with ALK-negative ALCL have a higher tendency to infiltrate the skin and the gastrointestinal tract. Involvement of other extranodal sites is less commonly seen. Primary
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Cutaneous ALCL is an unrelated disease with a different pathogenesis and clinical behaviour and without ALK expression and/or translocation, which is considered part of the spectrum of CD30+ T-cell lymphoproliferative disease of the skin. Nevertheless, there are rare bona-fide cases of primary cutaneous ALK-positive ALCL in pediatric and adult patients with long-term follow-up (32, 33).

The prognosis of ALK-positive ALCL is favourable compared with patients with other types of T-cell lymphomas, including ALK-negative ALCL (28). Approximately 90% of patients achieve complete remission with chemotherapy (30). However, the 2-year relapse rate of children with ALCL still reaches 30% after use of intensive chemotherapy strategies. Visceral involvement (liver, spleen and lung), skin lesions and a mediastinal mass increase the risk of progression or relapse (30). Interestingly, it has been shown that the small cell and lymphohistiocytic variants observed in around 15-30% of ALCL cases are significantly associated with a higher incidence of skin lesions and mediastinal involvement, and both variants showed a significantly higher risk of treatment failure in multivariate analysis, controlling for clinical characteristics (34). According to the International T-cell Lymphoma Project, the 5-year failure-free survival for ALK-positive ALCL is 60% versus 36% for ALK- negative ALCL, whereas the 5-year overall survival was 70% versus 49%, respectively (28). However, these differences could be explained by the fact that the majority of ALK-positive cases occur in the pediatric population, and if adjusted by age, no significant difference in failure-free survival or overall survival is evident (28).

6. IMMUNOPHENOTYPE

Immunohistochemical analysis is extremely useful in the correct diagnosis of ALCL, especially in the small cell and lymphohistiocytic variants. By definition, ALCL cells are strongly positive for CD30 in a membrane and Golgi pattern (figure 4A). However, CD30 expression is not specific for ALCL and can be seen in activated lymphoid cells, other T and B cell lymphomas, classic Hodgkin lymphoma and even in non-lymphoid neoplasias like embryonal carcinoma. In the small-cell variant, large cells show strong CD30 expression, while smaller cells are heterogeneous weakly positive (magnification x 40). C. ALK1 staining in a NPM-ALK-positive ALCL: neoplastic cells exhibit a cytoplasmic, nuclear and nucleolar staining (magnification x100). D. ALK1 staining in a TFG-ALK-positive ALCL: staining is restricted to the cytoplasm. E. ALK1 staining in small-cell variant ALCL: small neoplastic cells show a restricted nuclear staining while scattered large cells are strongly ALK positive (magnification x100). F. CD3ε staining in small-cell variant ALCL: the CD3ε reactivity of neoplastic cells is difficult to assess because they are surrounded by numerous non-neoplastic CD3ε-positive T lymphocytes (magnification x40). G. Double immunostaining using CD3ε (blue) and ALK (brown) antibodies reveals that ALK-positive neoplastic cells are CD3ε negative. Of note, neoplastic cells in small-cell and lymphohistiocytic variants are often concentrated around vessels (magnification x 40). H. ALCL usually shows a cytotoxic phenotype, demonstrated here with perforin (magnification x 40).
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restricted staining pattern, while rare cases associated with the CLTC-ALK translocation show a unique granular ALK cytoplasmic staining pattern (37, 38).

In the small cell variant of ALCL, and to a lesser extent in the lymphohistiocytic variant, scattered large cells are strongly ALK positive and small cells show a restricted nuclear staining (figure 4E).

A differential diagnosis of ALK+ ALCL is ALK+ large B-cell lymphoma, which shows plasmablastic features with CD20 negativity and cytoplasmic Ig expression (usually IgA), lacks CD30 expression and frequently shows CLTC-ALK fusions with granular ALK staining pattern, in rare cases also a classic NPM-ALK fusion (39-42).

ALK-positive ALCLs express one or more T-cell or NK-cell antigens but loss of several pan T-cell antigens is frequently observed resulting in a "null"-cell phenotype. CD3ε is negative in more than 75% of cases (24, 28, 43). However, this immunostaining can be difficult to interpret, namely in the small cell and lymphohistiocytic variants. In these tumors, neoplastic cells are rare and often surrounded by non-neoplastic CD3ε-positive T lymphocytes (figure 4F). Interestingly, the German Berlin-Frankfurt-Muenster (BFM) group reported that autologous hematopoietic stem cell transplantation (HSCT) is an efficient consolidation for patients suffering from relapsed CD3-negative ALCL but not for those with CD3-positive tumors (44). In such cases, a double staining using ALK1 and CD3 antibodies (figure 4G) could be useful. CD2, CD5 and CD4 are positive in a significant proportion of cases (70%). CD8 is usually negative, but rare CD8-positive cases exist especially in the small cell variant (32). Despite the usual CD4+/CD8- phenotype most cases express cytotoxic associated-antigens TiA1, granzyme B and perforin (figure 4H). The tumor cells are also positive for the interleukin 2 receptor, as demonstrated by the positivity with the CD25 antibody. CD15 expression is rarely observed as well as aberrant expression of PAX5, but when present might represent a diagnostic challenge in ALCL with "Hodgkin-like pattern" (45) ALK-positive ALCLs are consistently negative for Bcl2 and Epstein-Barr virus (EBV). Other markers characteristic of ALCL include clusterin, a marker of follicular dendritic cells (46) and the transcription factor C/EBPB a master regulator of macrophage differentiation (19).

6.1. T-cell receptor (TCR) rearrangements, T-cell receptor expression and T-cell receptor signalling

Characterization of T-cell identity and T-cell features of ALK-positive ALCL neoplastic cell was a long evolutionary process. Although it was already recognized in early years that ALK-positive ALCL shows loss of pan-T-cell antigens (null phenotype) or express only one or few T-cell markers (12, 24), molecular studies demonstrated; nevertheless, that ALCL was a neoplasia of T-cell origin and not of histiocytic origin, as it was originally thought (9). TCR rearrangements were first studied systematically in 1987 (47) and monoclonal TCR patterns have been verified in several studies since then (43, 48). Interestingly, although clonal TCR rearrangements have been shown in 74-90% of ALK-positive ALCL cases by sensitive PCR methods (43, 49) a loss of TCR proteins and proximal TCR signaling molecules in tumor cells were observed revealing a very characteristic feature of this disease. The loss of the TCR molecules, CD3 and ZAP-70 was first described in 2004 (43), followed by studies showing broader defects in T-cell signaling cascades, including reduced expression of LAT and SLP76 (50).

Efficient T-cell reactions upon ligation of MHC molecules require interaction of the TCR/CD3 complex with the co-receptors CD4 or CD8 and close proximity of CD45. In consequence receptor-associated tyrosin kinases LCK and FYN are activated and subsequently further transduction molecules like ZAP-70, LAT and SLP76 transmit downstream signaling (figure 5) (51). ALCL cells phenotypically represent activated T-cells that normally undergo activation-induced cell death, implying that the loss of TCR signaling molecules likely provides a biological advantage to ALCL cells. The precise mechanisms leading to the loss of T-cell identity in ALK+ ALCL is not fully understood; however, there seem to be different mechanisms involved including epigenetic silencing of key T-cell transcription factors (TCF1, LEF1 and GATA3) (52).

7. GENETIC FEATURES

The characteristic genetic alteration in ALK-positive ALCL is the ALK translocation, which was first identified in 1994 (2). Besides the main translocation t(2;5) (p23;q35), which fuses the ALK tyrosine kinase domain at 2p23 to the NPM gene at 5q35 in about 80% of cases, a growing number of different translocation involving the ALK locus have been identified. ALK translocations are the critical alteration, leading to constitutive activation of the ALK-tyrosine kinase, believed to promote tumorigenesis (53). ALK stimulates proliferation and survival of ALCL cells through the activation of different pathways including JAK/STAT, MAPK and PLCG signaling pathways. Additionally, the activated STAT3 is able to induce the expression of transcription factors like C/EBPB or AP-1 (overview see (54)). Secondary chromosomal alterations have been found in 58% of ALK+ ALCL cases, including recurrent gains of 17p and 17q24 and losses of 4q13-q21, and 11q14 (55).

8. TREATMENT

The current treatment approach for ALCL is based on standard frontline and salvage chemotherapy regimens designed for diffuse large
B-cell lymphoma. Most patients are treated with anthracycline containing chemotherapy regimens such as CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone). High-intensity regimens have not shown any benefit over standard-dose regimens (56). The unique phenotype and molecular features of ALCL make this disease an ideal model for developing targeted therapies. Brentuximab Vedotin is a novel anti-CD30 monoclonal antibody conjugated to the antimicrotubule cytotoxin monomethyl auristatin-E. It has been used to treat patients with relapsed/refractory CD30+ lymphomas. In the phase I trial, only two patients with ALK-positive ALCL were enrolled, both achieved complete remission (CR) (57). In a phase II trial for refractory or relapsed ALCL (28% ALK-positive; 72% ALK-negative), 57% achieved CR with a median response duration of 13 months. Median PFS and duration of response were not different according to ALK status (58). Brentuximab Vedotin has been approved by the Federal Drug Administration (FDA) for the treatment of systemic ALCL after failure of at least one chemotherapy regimen. However, right now there are ongoing trials with Brentuximab Vedotin combined with first-line therapy regimens like CHOP or CHP as frontline treatment of ALCL and other CD30+ haematological malignancies.
Crizotinib was the first ALK inhibitor that entered clinical practice and is currently used primarily in ALK-positive non-small cell lung carcinoma (NSCLC). Crizotinib has also been used to treat ALCL refractory to chemotherapy. The first two described cases displayed spectacular response to the ALK inhibitor achieving CR (22). A follow-up study from the same group confirmed the efficacy of crizotinib in additional 11 patients with advanced, resistant ALK-positive lymphomas (59). Similar results have been reported by other groups (60). Crizotinib has been used as a bridge therapy to allogeneic stem cell transplantation (SCT) in refractory ALK-positive ALCL and provided maintenance in post-allo STC with promising effects (61). The impressive results obtained with this novel targeted therapy have made a huge impact in routine clinical practice, and is likely to change the treatment paradigm in ALCL.

9. CONCLUSIONS AND PERSPECTIVES

The last 20 years, since the discovery of the NPM-ALK fusion, which consolidated a morphologically and phenotypically characterized lymphoma subtype into a molecularly defined clinico-pathological entity, have seen spectacular progress in our understanding of the biological basis of this disease. The discovery of the translocation and cloning of the ALK gene on chromosome 2p23 led to further definition of the ALCL morphological spectrum with recognition of the small cell and lymphohistiocytic variants. With the advent of targeted therapies against CD30 and ALK, cross-fertilized by therapeutic needs in other tumor entities such as NSCLC, we likely will see a further improvement in the already excellent cure rates for patients with ALK+ ALCL, potentially accompanied by a reduction in cytotoxic drugs and associated side effects. Irrespective of the ongoing clinical progress, this lymphoma remains an excellent example of the power of morphology in characterizing a disease entity that ultimately led to the discovery of the genetic alteration. ALK+ ALCL in 2015 is a model for a malignant disease driven by the effects of a dominant oncogene. Experimental results obtained in ALK+ ALCL are likely to provide benefit not only for the patients suffering of this disease but for research in other cancer types.

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