Anaplastic large cell lymphoma: changes in the World Health Organization classification and perspectives for targeted therapy

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Anaplastic large cell lymphoma (ALCL) was first described in 1985[1] as a neoplastic proliferation of lymphoid cells that are anaplastic in appearance (Figure 1A), have a propensity to grow cohesively, invade lymph node sinuses (Figure 1B) and consistently express the CD30 molecule.[2] Major breakthroughs came with the discovery that some ALCL tumors carried the t(2;5) translocation which caused fusion of the nucleophosmin gene (*NPM1*) with a previously unrecognized gene, named anaplastic lymphoma kinase (*ALK*).[3] Since then, a large bulk of information has accumulated on the role of ALK in the molecular pathogenesis of ALCL[4].

Notable progress was also made in characterizing ALCL with the generation of specific monoclonal antibodies that permitted immunohistochemical detection of ALK chimeric proteins directly on fixed paraffin-embedded biopsy samples.[5,6] These reagents aided in clarifying controversies over the wide morphological spectrum of ALCL and in better defining the borders between ALCL and other lymphoma subtypes, such as Hodgkin's lymphoma and peripheral T-cell lymphoma (PTCL).

ALCL was first included as an entity in the Revised European and American Lymphoma (REAL) classification[7] in 1994, and, subsequently, in the World Health Organization (WHO) classification of lymphoid neoplasms in 2001. The new edition of the WHO classification (2008) recognizes, within the spectrum of mature T-cell neoplasms, two types of systemic ALCL according to ALK protein expression in tumor samples:[8] (i) a distinct entity, named ALK$^+$ ALCL, which is characterized by *ALK* gene rearrangements and ALK protein expression; and (ii) a provisional entity, the so-called ALK$^-$ ALCL, which cannot be distinguished morphologically from ALK$^+$ ALCL but differs from this entity because of the lack of ALK protein.

Diagnosis of anaplastic large cell lymphoma

ALK-positive anaplastic large cell lymphoma

ALK$^+$ ALCL, thought to derive from an activated mature cytotoxic T cell, is genetically the only well-characterized entity in the spectrum of mature T-cell neoplasms.[8] Neoplastic transformation is due to constitutive expression and activation of oncogenic ALK fusion proteins deriving from *ALK* gene rearrangements.[4] *ALK* fuses with the *nucleophosmin (NPM)* gene in about 85% of case of ALCL and with a partner other than *NPM* in the other cases[9] (Figure 2). ALCL cases expressing NPM-ALK or an ALK-variant fusion protein are distinguished by subcellular distribution of ALK protein. In fact, when an NPM-ALK protein is present, lymphoma cells show positivity for ALK in the cytoplasm and nucleus (Figure 1C) while, in the presence of an ALK variant fusion protein, expression of ALK is

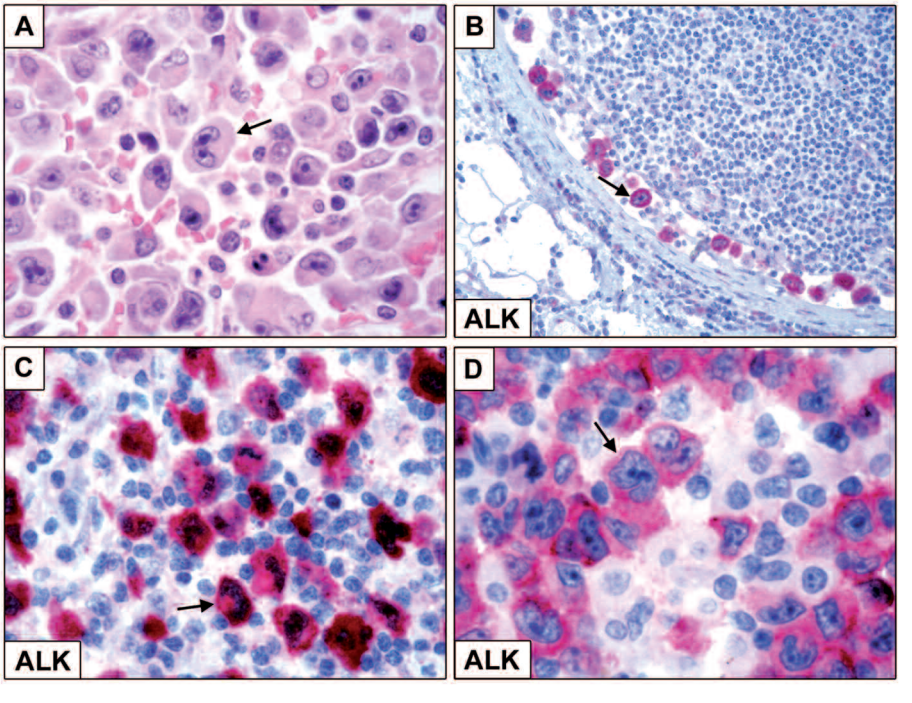

Figure 1. (A) **Morphological appearance of ALK$^+$ anaplastic large cell lymphoma (ALCL) of common type (hematoxylin-eosin; x 800). The arrow indicates a typical hallmark tumor cell. Cases with the same morphological features but lacking the ALK protein are classified as ALK-negative ALCL.** (B) **Minimal lymph node involvement with an intrasinusoidal pattern in ALK$^+$ ALCL. The arrows points to an ALK$^+$ lymphoma cell within the subcapsular sinus (x 200).** (C) **In ALCL carrying the NPM-ALK fusion protein, positivity for ALK (in red) is found both in the cytoplasm and nucleus of neoplastic cells (x 800, arrow).** (D) **In ALCL carrying a ALK variant fusion protein, positivity for ALK (in red) is restricted to the cytoplasm of neoplastic cells (x 800, arrow). B-D: immunostaining of lymph node paraffin sections with anti-ALK monoclonal antibody (clone ALKc); APAAP technique and hematoxylin counterstaining.**

restricted to tumor cell cytoplasm[10,11] (Figure 1D). Interestingly, some fusion proteins show unique ALK subcellular expression patterns.[11] In particular, ALK positivity in ALCL carrying clathrin-ALK and moesin/ALK is granular cytoplasmic and surface membrane, respectively.

Overlapping clinico-pathological features[10] and gene expression profile[12] justify inclusion of ALCL expressing NPM-ALK or ALK variant fusion proteins under the single term of ALK$^+$ ALCL. This disease entity is characterized by a broad morphological spectrum, including common type, lympho-histiocytic, small-cell and Hodgkin-like variants.[8] Recognizing these morphological patterns is crucial as they can mimic both inflammatory processes and malignant tumors other than ALCL. For example, in the lympho-histiocytic variant, abundant histiocytes frequently mask the tumor cell population and may lead to an incorrect diagnosis of atypical inflammatory lesions or hemophagocytic syndrome.[8] On the other hand, the small-cell variant, due to the predominance of the small cell component, may be misdiagnosed as PTCL not otherwise specified (PTCL-NOS)[8]. Under all these circumstances, determining ALK protein expression establishes the correct diagnosis.

ALK-negative anaplastic large cell lymphoma

In the 2008 WHO classification, ALK-ALCL is recognized as a new provisional entity within the spectrum of mature T-cell neoplasms.[8] ALK-ALCL, the genetic underlying lesion(s) of which remains unknown, is defined as a CD30$^+$ T-cell lymphoma that, despite being morphologically indistinguishable from ALK$^+$ ALCL, lacks the ALK protein.[8]

Thus, major criteria for diagnosing ALK$^-$ ALCL are morphological features and strong CD30 expression by all neoplastic cells. ALK$^-$ ALCL differs from PTCL-NOS[8] because of: (i) absence of small-to-medium sized neoplastic lymphocytes (frequently found in PTCL-NOS); (ii) strong, homogeneous CD30 expression (variably expressed in PTCL-NOS); and (iii) lack of T-cell receptor proteins (usually retained in PTCL-NOS). However, the distinction is not always straightforward and even expert hemopathologists may disagree on this topic.[8]

Immunophenotypic and genetic studies allow a distinction of ALK$^-$ ALCL from tumor cell-rich classic Hodgkin's lymphoma to be made in virtually all dubious cases. According to the 2008 WHO classification,[8] the most important diagnostic criteria favoring a diagnosis of ALK$^-$ ALCL are: (i) nuclear negativity for the PAX5 transcription factor (usually expressed in classic Hodgkin's lymphoma); (ii) negativity for the EBV markers EBER and LMP1 (which may be expressed in classic Hodgkin's lymphoma); and (iii) presence of clonal T-cell receptor rearrangements (usually absent in classic Hodgkin's lymphoma).

Clinical features, therapy and prognosis of anaplastic large cell lymphoma

Separation of systemic ALCL into two entities (ALK$^+$ and ALK$^-$)[8] is clinically and prognostically relevant. ALK$^+$ ALCL mostly occurs in the first three decades of life while patients with ALK$^-$ ALCL are older.[13,14] Both forms

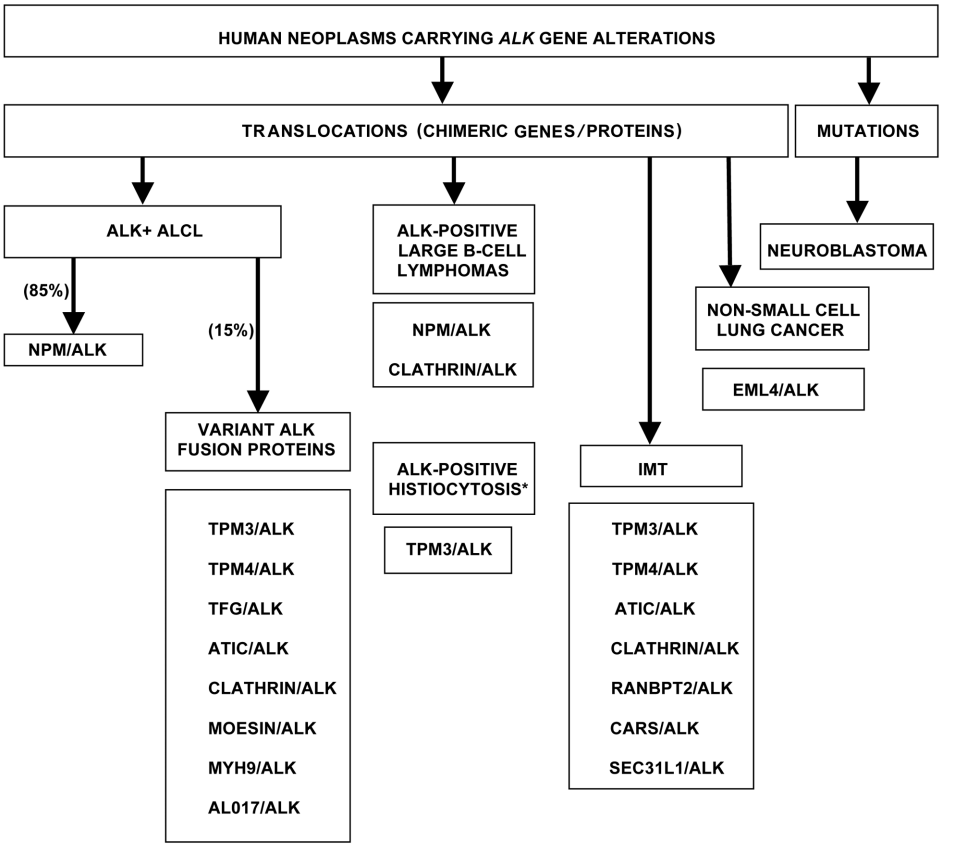

Figure 2. Alterations of the *ALK* gene (translocations or mutations) are found in a large variety of human neoplasms including hematopoietic tumors such as ALK$^+$ ALCL and ALK$^+$ diffuse large B-cell lymphoma (both included as distinct entities in the 2008 WHO classification), a small subset of non-small cell lung cancer carrying the *EML4-ALK* rearrangement and neuroblastoma, which may harbor *ALK* gene mutations. IMT indicates inflammatory myofibroblastic tumor. ALK expression in IMT usually occurs in young patients. * Three cases of ALK$^+$ histiocytosis have been reported. This entity occurs in early infancy and is characterized by a good outcome (Chan JK, Blood, 112:2965, 2008).

frequently present with B symptoms and stage IV disease, extranodal involvement being mainly observed in skin, bone, and soft tissues. Bone-marrow involvement is best identified by immunostaining for ALK protein.[8] Prominent leukemic involvement in ALK$^+$ ALCL is usually associated with the small cell morphological variant and carries a poor prognosis. The central nervous system is rarely affected in patients with ALK$^+$ ALCL.

Patients with ALCL generally receive the same treatment (CHOP-based regimens) as prescribed for diffuse large B-cell lymphoma. About 80% of children and about 60% of adults with ALK$^+$ ALCL are cured by chemotherapy, while patients with ALK$^-$ ALCL appear to have a poorer outcome.[13,14] According to a recent study,[15] age could be a prominent factor driving the difference in outcome, since no difference emerged in survival of ALK$^+$ and ALK$^-$ ALCL patients under 40 years of age. The International Prognostic Index (IPI) continues to maintain its prognostic value even in the good prognostic category of ALK$^+$ ALCL[13.] ALK$^-$ ALCL seems to have a better clinical outcome than PTCL-NOS[15], but further studies are required to confirm these findings.

Since ALK$^+$ ALCL is characterized by a good response rate and survival, consolidation with high dose therapy followed by autologous stem cell (ASCT) support is not recommended if patients achieve complete remission. This treatment option should probably be offered to ALK$^-$ ALCL patients with at least two IPI adverse prognostic factors, because of their poorer prognosis. At relapse, ASCT should be proposed to patients who are chemosensitive to salvage chemotherapy. However, this procedure is probably less efficient in ALK$^-$ ALCL than in ALK$^+$ ALCL.[16] Allogeneic transplantation was reported to be an effective procedure for relapsed or refractory ALK$^+$ ALCL[17] but its value in the treatment of ALK$^-$ ALCL remains to be defined.

Future perspectives

In the near future, it will be essential to look at the efficacy of innovative forms of therapy for high IPI or relapsed ALK$^+$ ALCL and for ALK$^-$ ALCL. Anti-CD30 monoclonal antibodies, whether native or conjugated to toxins or radioisotopes, may provide a new basis for the treament of ALCL,[2,18] especially the ALK-negative forms. Small molecules which are able to inhibit ALK kinase activity[19,20] are likely to play an important role in future therapy of ALK$^+$ ALCL. These compounds can inhibit ALK activity through two different mechanisms: (i) by competing with ATP for binding to the ALK kinase domain;[21] and (ii) by increasing the proteasome-mediated degradation of the oncogenic ALK fusion protein; examples of small molecules exhibiting this activity include geldanamycin, 17-allylamino-17-demethoxy-geldanamycin[22] and herbimycin A.[23] ALK inhibitors have shown strong activity against ALK$^+$ ALCL cells *in vitro* and in xenotransplanted ALK$^+$ ALCL tumors.[21] Another possible approach is to interfere with the ALK downstream signaling pathways.[24] The study by Bonvini *et al*.[25] published in this issue of the journal, moves in this direction as it demonstrates, for the first time, that targeting cyclin-dependant kinases (cdk) with flavopiridol is active against ALCL cells. Interestingly, response to flavopiridol increased when this drug was combined with ALK inihibitors which interrupted NPM-ALK signaling. Proteome identification of novel binding partners of the NPM-ALK fusion protein, using mass spectrometry, is expected to further expand our knowledge of altered signaling pathways in ALCL and provide new research directions for the development of targeted therapies.[26,27] At present, there are no small-molecule inhibitors of ALK approved for clinical use. However, since the ALK inhibitors investigated so far have shown considerable anti-tumor activity with minimal toxicity, it is expected that these compounds may enter clinical use in the next few years. The hope for the future is that the incorporation of ALK inhibitors in treatment schedules for ALK$^+$ ALCL may result in even better clinical outcomes with fewer toxic effects.

ALK$^+$ neoplasms other than ALCL could also benefit from novel therapies. One potential candidate is the rare form of diffuse large B-cell lymphoma expressing the ALK protein[28] (Figure 2), which the WHO classification has now recognized as a new entity. This tumor is characterized by immunoblastic/plasmoblastic (rather than anaplastic) morphology, cytoplasmic expression of IgA, negativity or weak positivity for CD30, and, usually, a poor outcome. Although *EML4-ALK* rearrangements were detected in non-small cell lung cancer[29] (Figure 2), their biological and clinical significance remains to be better defined.[30,31] Clinical trials testing the efficacy of ALK inhibitors against this subset of lung tumors are ongoing and should soon provide answers. Assessment of the activity of ALK inhibitors against neuroblastomas harboring *ALK* gene mutations is also warranted.

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