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CD30⁺ lymphoproliferative disorders

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CD30 antigen, originally identified as a cell surface marker of the malignant Hodgkin and Reed-Sternberg (HRS) cells in Hodgkin's lymphoma by the use of the Ki-1 monoclonal antibody, is a transmembrane glycoprotein member of the tumor necrosis factor (TNF) receptor superfamily.[1] In lymphoid cells, CD30 is an activation marker inducible *in vitro* by mitogenic signals and viral stimulation, and its expression is detected in a small number of immunoblasts in benign lymphatic tissues.[2] In pathological conditions, CD30 is found at variable levels in different lymphomas of B-cell or T-cell derivation, and in several reactive conditions (Table 1). However, strong and homogeneous CD30 expression in most neoplastic cells is restricted to fewer entities, mainly three groups of lymphoid neoplasms: (i) classical Hodgkin's lymphoma, (ii) anaplastic large cell lymphomas (ALCL), and (iii) primary cutaneous CD30⁺ T-cell lymphoproliferative disorders.[3] For diagnostic purposes, the detection of CD30 is of particular value as a hallmark feature, albeit not specific, for the identification of these entities.

The disorders most characteristically associated with CD30 are distinct clinico-pathological entities - interestingly with some morphological similarity, as 'Hodgkin's-like' features may be encountered in both ALCL and primary cutaneous CD30⁺ lymphoproliferative disorders. Although there has been a lot of speculation in the past about the relationship and possible overlap between classical Hodgkin's lymphoma and ALCL, it is now clear that these are biologically distinct entities of different cellular derivation (B-cell *versus* T-cell, respectively). Historically, CD30 was instrumental in identifying ALCL as lymphomas composed of large cells showing homogeneous expression of CD30 at high levels, and characterized by cohesive growth and peculiar 'anaplastic' cytomorphological features.[4] Among these, a small subset of cases of B-cell derivation represent variants of diffuse large B-cell lymphoma. Nowadays, the designation ALCL is restricted to cases of T-cell derivation. These overall infrequent neoplasms involving lymph nodes and/or extranodal sites comprise so-called typical 'hallmark cells' - characterized by an eccentric horseshoe-shaped nucleus and a prominent eosinophilic Golgi region. Anaplastic lymphoma kinase (*ALK*) gene status was found to be another critical parameter to characterize two subsets of ALCL.[5] Molecularly defined ALK-positive ALCL is mostly a disease of children and young adults, carries a relatively good prognosis and comprises a morphological spectrum including variants deviating from the common type by the presence of only occasional 'hallmark' tumor cells and/or an associated reactive background. Conversely, ALK-negative ALCL affects older individuals and is associated with a worse prognosis, closer to that of peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS).[6] The view of ALK-positive and ALK-negative ALCL as two variants of the same entity evolved towards the concept of two separate disease entities in the current WHO classification of hematologic malignancies.[3] Although the majority of ALCL occur as primary systemic disorders, a subset of ALK-negative ALCL – referred to as primary cutaneous ALCL - occurs primarily as single or multifocal tumor lesions in the skin, usually remains localized to the skin, may undergo spontaneous regression and generally has a favorable prognosis. Because of overlapping clinical and pathological features with lymphomatoid papulosis, a clinically benign recurring skin lymphoproliferative disease composed of large atypical 'anaplastic' CD30⁺ cells admixed with an inflammatory background, both primary cutaneous ALCL and lymphomatoid papulosis are considered within the spectrum of primary cutaneous CD30⁺ T-cell lymphoproliferative disorders. Figure 1 provides a synoptic view of CD30⁺ lymphoproliferations of T-cell derivation.

A peculiar feature of ALCL is that, despite the presence of monoclonal T-cell receptor (*TCR*) gene rearrangement indicative of T-cell lineage derivation, its manifestations of a T-cell immunophenotype are usually limited. Indeed, ALCL tumor cells usually show reduced or absent expression of one or more T-cell antigens or

may even have an apparent 'null cell' phenotype, with the most commonly preserved antigens being CD2, CD4 and CD45.[6,7] The usual negativity for CD3 has been a focus of interest given its potential functional consequences, since CD3 molecules are associated with the TCR and transduce the signal of TCR engagement to ZAP-70 tyrosine kinase. In 2004, Bonzheim *et al.* from Würzburg showed that ALCL lack expression of TCR molecules and have markedly reduced or absent expression of ZAP-70.[8]

In this issue of the journal, Geissinger *et al.* expand their previous work and report on the disturbed expression of the TCR/CD3 complex and associated signaling molecules in CD30$^+$ T-cell lymphoproliferations.[9] The analysis was conducted by immunohistochemistry on a large series of tissues comprising 71 cases of systemic ALCL (33 ALK-positive and 38 ALK-negative) and 19 primary cutaneous CD30$^+$ lymphoproliferative disorders (10 cases of primary cutaneous ALCL and 9 of lymphomatoid papulosis) in comparison to 20 cases of PTCL, NOS. They found random losses in various combinations of the TCRα/β and the four CD3 subunits (γ, δ, ε and ζ chains) in most cases of systemic and cutaneous CD30$^+$ lymphoproliferative disorders, contrasting with the homogeneous expression in most cases of PTCL, NOS. Regarding the TCR signaling pathway, in addition to ZAP-70, several other downstream mediators of the pathway (Lck, LAT and NFATc1) were down-regulated in CD30$^+$ T-cell lymphoproliferations. Within the whole group of CD30$^+$ T-cell lymphoproliferations, there was a tendency for a continuum of abnormalities which were, overall, maximal in ALK-positive ALCL, intermediate in ALK-negative ALCL and partial in primary cutaneous CD30$^+$ lymphoproliferative disorders. The applicability of these markers in hematopathology practice may be hampered by the complexity of the antibody panel and variability of the expression patterns. Although the data presented suggest that loss of the T-cell phenotype is specific to ALCL rather than PTCL, NOS, the diagnostic value of this feature remains to be defined by focusing the analysis on cases with diagnostically challenging borderline features.

The characterization of T-cell identity loss as a feature shared by CD30$^+$ lymphoproliferations expands the recent documentation, by Ambrogio *et al.*, of an extensive loss of T-cell-specific molecules, including CD3ε, ZAP-70, LAT and SLP76, related to TCR signaling in systemic ALCL.[10] Interestingly, this is also in line with genome-wide expression profiling studies in which fairly similar molecular signatures and pathways have been found for systemic ALCL irrespective of *ALK* gene rearrangements,[11-13] while few differences have been found between cutaneous and systemic cases of ALCL.[14] In particular, the level of similarity observed between the neoplastic cells in systemic ALK-negative ALCL and primary cutaneous CD30$^+$ lymphoproliferations is noteworthy as these conditions usually have clearly different clinical presenting features and evolution. A possibility is that, despite similar molecular profiles, distinct signaling pathways are activated in the different entities, and/or triggering of similar pathways may induce distinct effects. For example the effects of CD30 stimulation by agonistic antibodies or its engagement with CD30-ligand have been shown to vary according to the cell type, as they are basically absent in Hodgkin's-like cells while inducing decreased proliferation in ALCL cells.[15,16] The specificity of the microenvironment linked to the anatomic site and/or that of the tumor-associated reactive cellular infiltrate may also be determinant in modulating the properties and growth of the neoplastic cells.

The association between strong CD30 expression and altered expression of T-cell-specific molecules in CD30$^+$ T-cell lymphoproliferations calls into question the possibility of a causal and/or functional relationship. Deregulated CD30 expression in ALCL has been linked to activation of transcription factors of the AP-1 family, including c-Jun and JunB.[17,18] Regarding the expression of transcription factors involved in the regulation of the TCR/CD3 complex in CD30$^+$ lymphoproliferations, Geissinger *et al.* identified some defects (involving especially TCF-1 and TCF-1α/LEF-1) in the whole group of CD30$^+$ cases; however, in the absence of clear-cut correlations at the level of single cases they concluded that transcriptional dysfunction is probably not the primary cause of TCR/CD3 loss.

In ALK-positive ALCL, experimental data provide evidence that both CD30 expression and down-regulation of T-cell molecules are regulated by NPM-ALK tyrosine kinase activity. The activation of CD30 transcription has been shown to be mediated by JunB, while TCR/CD3 silencing has been linked to NPM-ALK-mediated STAT3 activation and involves epigenetic silencing by hypermethylation.[10,19] For other CD30$^+$ T-cell lymphoproliferations the primary oncogenic alterations are unknown, and it is unclear whether deregulation of CD30 and TCR/CD3 are linked to a common aberration or occur independently.

The characteristic loss of T-cell-specific molecules in a subset of peripheral T-cell neoplasms provides a new direction for further investigations. It will be of interest to examine the biological and functional consequences of

Table 1. Lymphoproliferations with CD30 expression.

Primary systemic
Classical Hodgkin's lymphoma*
Anaplastic large cell lymphoma, ALK-positive*
Anaplastic large cell lymphoma, ALK-negative*
Subsets of diffuse large B-cell lymphoma
Anaplastic variant Primary mediastinal large B-cell lymphoma Primary effusion lymphoma
Subsets of peripheral T-cell lymphoma entities
EBV-positive B-cell lymphoproliferative disorders
Reactive lymphoid hyperplasias
Primary cutaneous
Primary cutaneous CD30-positive T-cell lymphoproliferative disorders*
- Primary cutaneous anaplastic large cell lymphoma* - Lymphomatoid papulosis*
Mycosis fungoides, large cell variant
Subsets of non-epidermotropic cutaneous lymphomas
Reactive cutaneous lymphoproliferations

**entities characterized by strong and homogeneous CD30 expression*

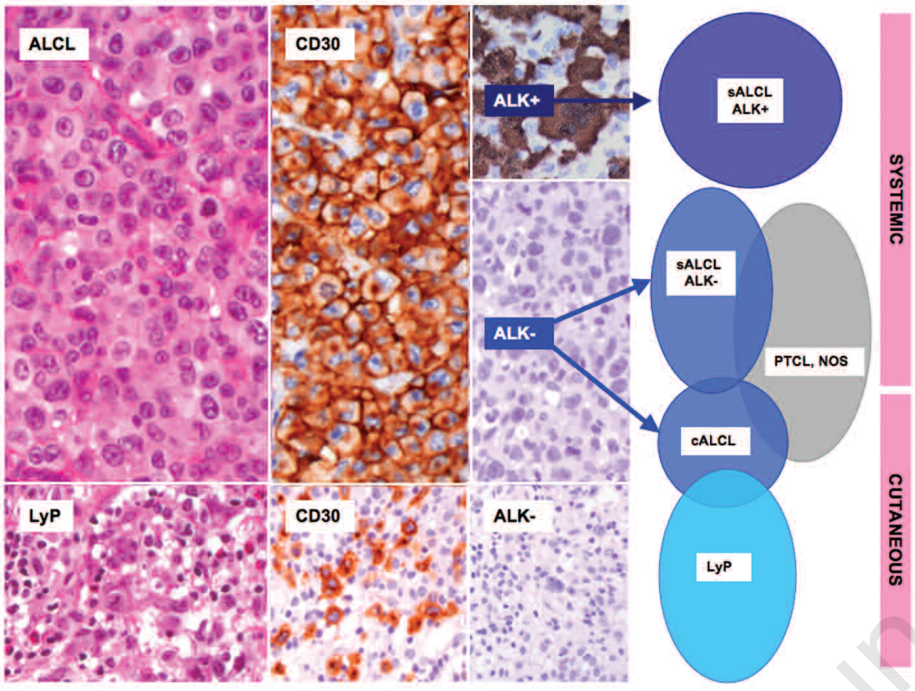

Figure 1. CD30⁺ T-cell lymphoproliferations.

ALCL comprises sheets of large cells, including hallmark cells with horseshoe-shaped nuclei and a paranuclear Golgi region. LyP comprises scattered large anaplastic cells in an inflammatory background. The overlaps between the entities featured on the right are: the interface between PTCL, NOS, and ALK-negative ALCL (primary systemic or cutaneous); the interface between sALCL and cALCL as sALCL may present in the skin and conversely primary cutaneous ALCL may disseminate systemically; the interface between cALCL and LyP as these represent a continuum.
ALCL: anaplastic large cell lymphoma; ALK: anaplastic lymphoma kinase; c: cutaneous; LyP: lymphomatoid papulosis; PTCL, NOS: peripheral T-cell lymphoma, not otherwise specified; s: systemic.

TCR/CD3 silencing. Since TCR silencing occurs as a downstream effect of the oncogenic tyrosine kinase activity in ALK-positive ALCL, it is tempting to speculate that it may be involved in the development of the malignant phenotype. Indeed, TCR silencing appears to be an early event in CD30⁺ T-cell lymphoproliferative disorders, as exemplified in lymphomatoid papulosis. Moreover, defects in CD3/TCR expression have been reported in other forms of T-cell malignancies, including angioimmunoblastic T-cell lymphoma and human T-cell lymphotropic virus-associated T-cell proliferations, as well as an early aberration in the clonal T-cell populations encountered in the lymphocytic variant of the hypereosinophilic syndrome.[20,21] Advances in the understanding of the cell functions deregulated by TCR silencing in these neoplasms are essential for assessing the potential therapeutic importance of restoring T-cell identity. Indeed, from a therapeutic standpoint, alternative treatments are needed for patients with relapsing or refractory forms of systemic ALCL, and a small subset of poorly controlled cutaneous CD30⁺ lymphoproliferations. The development of monoclonal antibodies targeting the CD30 molecule constitutes a promising approach in such patients and could be combined with other innovative treatment modalities such as inhibitors of ALK activity for ALK-positive tumors.[22,23]

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Prophylaxis of invasive fungal diseases in patients with hematologic disorders

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Invasive fungal diseases are associated with significant morbidity and mortality among neutropenic patients after chemotherapy and in allogeneic hematopoietic stem cell transplant (HSCT) recipients. Considering that it is difficult to make an early diagnosis, the prophylaxis of these complications is appealing. Prevention strategies are based on environmental precautions and antimicrobial agents. While there is a general agreement on the role of air filtration in the control of airborne filamentous fungal infections, the indication for pharmacological prophylaxis is still debated.[1]

Until a few years ago, only fluconazole and itraconazole had been evaluated in randomized, controlled trials for primary antifungal prophylaxis in patients with hematologic disorders.[2-4] In view of the results of these studies, international guidelines did not recommend primary antifungal prophylaxis for all neutropenic patients, including those who had undergone autologous HSCT or had acute leukemia, and only recommended prophylaxis of *Candida* infections with oral or intravenous fluconazole for allogeneic HSCT recipients during the period of neutropenia until engraftment.[5-7]

In recent years, awareness of the epidemiological impact of invasive aspergillosis and less common molds, including zygomycetes, *Fusarium* species and *Scedosporium* species, has increased worldwide. At the same time, new broad spectrum and well tolerated antifungal drugs, in particular second generation triazoles (posaconazole and voriconazole) and echinocandins, became available, and prospective, controlled trials have been conducted to investigate their ability to prevent invasive fungal infections in high-risk hematologic patients.[8-10] Based on the above studies, current international guidelines continue to recommend the use of fluconazole until engraftment in patients who have undergone allogeneic HSCT, and, for the first time, recommend the use of a broad spectrum drug, oral posaconazole, during intensive immunosuppressive therapy for graft-*versus*-host disease, and in patients with acute myeloid leukemia or myelodysplastic syndromes during remission induction chemotherapy.[11-13] These recommendations reflect important progresses obtained in the prevention of invasive fungal infections, including those caused by filamentous fungi, but they have been unable to generate a consensus on the optimal prophylaxis of invasive fungal infections in the complex scenario of hematologic disorders, particularly in the transplant setting. This problem has been underlined in a recent consensus process by the *Gruppo Italiano Trapianto di Midollo Osseo* (GITMO) which observed that key recommendations by international guidelines imply various problems such as the lack of any approved mold-active prophylaxis during the engraftment phase in allogeneic HSCT, and the lack of an intravenous formulation of posaconazole, which could limit the use of this drug in patients unable to tolerate oral medications or with altered intestinal absorption.[14] Based on the preliminary results of two controlled studies of primary prophylaxis with voriconazole in allogeneic HSCT recipients during engraftment and graft-*versus*-host disease phases, and pending publication as full papers, the 2009 updated European guidelines (ECIL 3) provisionally recommended the use of voriconazole in both phases of HSCT.[13]

An evidence-based approach to secondary antifungal prophylaxis in patients with a previous invasive fungal infection who require further antileukemic treatment remains even more challenging. An anti-infection strategy