CD30 expression in peripheral T-cell lymphomas

CD30 antigen is a trans-membrane glycoprotein belonging to the tumor necrosis factor receptor superfamily.[1] Upon stimulation, CD30 exerts pleiotropic effects on cell growth and survival, which largely depend on the NF-κB pathway activation.[2] In normal or inflamed tissues, CD30 expression is restricted to medium/large activated B- and/or T- lymphocytes,[1,3] while among lymphoproliferative disorders (LPDs) it was initially reported in classical Hodgkin's lymphoma (cHL) and anaplastic large cell lymphoma (ALCL).[4] The specific and highly dense CD30 expression on the lymphomatous cells makes it an attractive target for drug-conjugated antibody-directed therapies, as first reported by Falini *et al*. in refractory cHL,[5] and later confirmed in experimental models on ALCL.[6] In recent years, the anti-CD30 compounds again attracted clinical interest for the availability of a monomethyl auristatin E-conjugated anti-CD30 antibody (Brentuximab Vedotin) which produced encouraging results in clinical trials on refractory/resistant cHL or ALCL patients.[7,8]

Regarding peripheral T-cell lymphomas (PTCL) CD30 expression was observed in a subset of primary cutaneous LPDs,[9] enteropathy associated T-cell lymphoma (EATL, type 1),[10] extranodal NK/T-cell lymphoma nasal type (ENTL),[11] mycosis fungoides (MF),[12] transformed MF (t-MF)[13] and peripheral T-cell lymphoma not otherwise specified (PTCL-NOS).[14] Given the extremely poor prognosis of PTCL and the current unavailability of effective therapies, we assessed CD30 expression in 192 PTCL at onset, in order to assess the feasibility of immune-therapy administration in such tumors. The formalin-fixed paraffin-embedded samples were retrieved from the archives of the Hematopathology Section, University of Bologna, Italy, and included: 42 angioimmunoblastic T-cell lymphomas (AITL), 41 MF (of which 9 t-MF), 12 EATL (n=9 type 1; n=3 type 2), 10 ENTL, 87 PTCL-NOS. Immunohistochemistry was performed by applying the standard reference antibody clone Ber-H2 on full sections. Heat/EDTA-based antigen retrieval methods and detection techniques such as EnVision™ FLEX Target Retrieval Solution, high pH and Dako REAL Detection System were used.[15] Double stain-

Table 1. CD30 immunohistochemical expression in PTCLs other than ALCL.

	CD30 IHC SCORE					
	0	1+	2+	3+	4	Score ≥2+
PTCL, NOS (87 cases)	31 (35.63%)	11 (12.64%)	18 (20.69%)	11 (12.64%)	16 (18.39%)	45/87 (51.72%)
AITL (42 cases)	24 (51.14%)	9 (21.42%)	5 (11.90%)	4 (9.52%)	–	9/42 (21.42%)
ENTL (10 cases)	2 (20.00%)	1 (10.00%)	3 (30.00%)	1 (10.00%)	3 (30.00%)	7/10 (70.00%)
MF (32 cases)	13* (40.63%)	15** (46.88%)	2§ (6.25%)	–	2§§ (6.25%)	4/32 (12.50%)
Transformed MF (9 cases)	–	–	3 (33.33%)	6 (66.67%)	–	9/9 100%
EATL type 1 (9 cases)	–	–	2 (22.22%)	–	7 (77.78%)	9/9 (100.00%)
EATL type 2 (3 cases)	3 (100%)	–	–	–	–	–
All types (192 cases)	73 (38.02%)	36 (18.75%)	33 (17.18%)	17 (8.85%)	28 (14.58%)	83/192 (43.22%)

*PTCL, NOS: peripheral T-cell lymphoma, not otherwise specified; AITL: angioimmunoblastic T-cell lymphoma; ENTL: extranodal NK/T-cell lymphoma, nasal type; MF: mycosis fungoides; EATL: enteropathy-associated T-cell lymphoma; *2 cases in tumoral phase; **1 case in tumoral phase; §follicolotropic variant; §§pagetoid reticulosis subtype.*

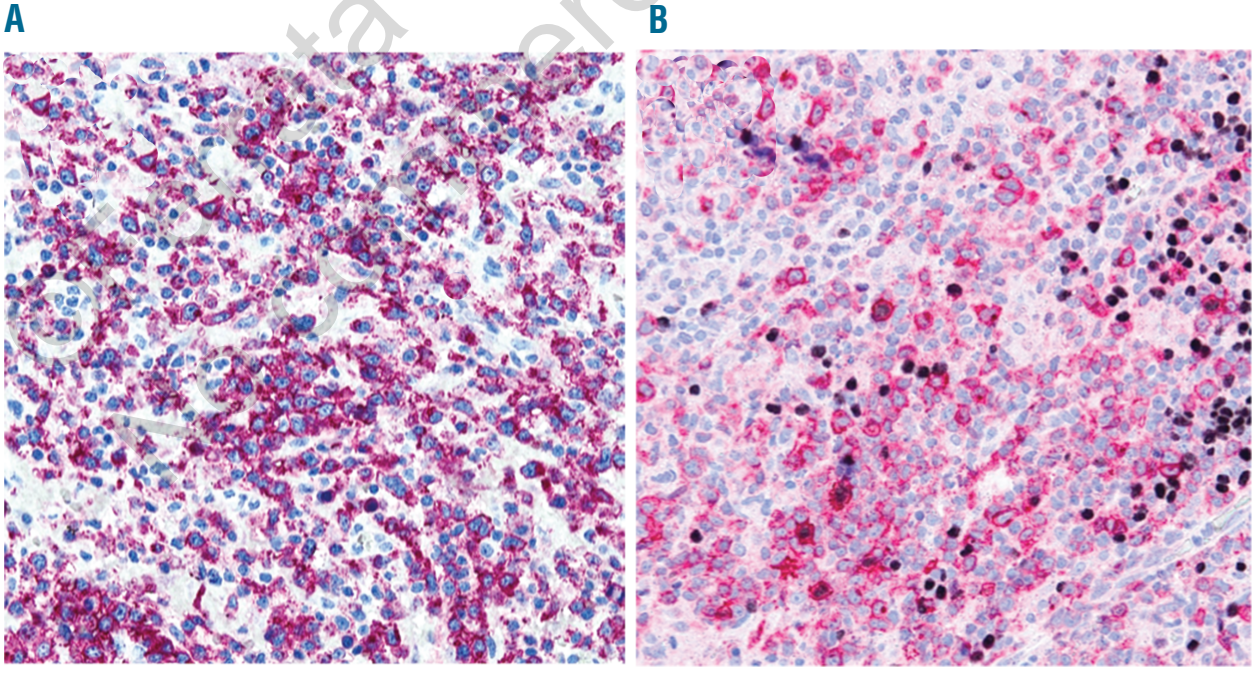

Figure 1. Representative examples of CD30 expression in peripheral T-cell lymphomas. (A). PTCL, NOS with high score (4+) CD30-expression (CD30 staining; Dako REAL Detection System; original magnification 20; 40x); (B). AITL with high score (3+) CD30-expression and scattered CD30-positive/PAX5-positive large B blasts (CD30 [red]; Dako REAL Detection System; PAX5 [brown]; EnVisionTM FLEX Target Retrieval Solution. Original magnification 20x).

ing for the nuclear B-cell associated marker PAX5 and the CD30 molecule was also performed in all AITL cases. The immunohistochemical results were scored by 2 pathologists (ES and SAP) on a 5-tiered scale (0 no staining; 1+ <25% positive cells; 2+ 25-50% positive cells; 3+ >50-75% positive cells; 4+ >75% positive cells). Diagnostic cells from cHL were used as positive controls. The staining intensity was graded as weak, moderate and strong. Overall, 43.22% (83 of 192) of the considered cases showed a CD30 expression score of 2+ or more (Table 1). The results with ENTL, EATL type 1 and 2 and t-MF are in keeping with previously reported data,[7-9] while those for PTCL-NOS (51.72%; 45 of 87) and AITL (21.42%; 9 of 42) are higher than those reported on tissue micro-arrays[14] (Figure 1). The results for AITL in Table 1 are based on PAX5/CD30 double staining and refer to the PAX5 negative/CD30 positive neoplastic cells. In comparison to the single anti-CD30 staining, that also included PAX5 positive/CD30 positive B blasts, only 5 cases were down-graded (n=3 cases from 4+ to 3+, n=2 cases from 2+ to 1+). Interestingly, a relatively high percentage of MF showed a moderate positivity for CD30 (score ≥2+ 12.50%, 4 of 32): in keeping with our data, similar results have been recently reported in the literature.[12] In all cases, staining intensity ranged from moderate to strong within the same section; the variability was often related to cell size, with small/medium cells showing moderate intensity and large cells a stronger one. No further correlation was found between CD30 expression and other morphological parameters.

From a clinical perspective, these data potentially include some PTCL in the spectrum of the LPDs suitable for anti-CD30 immunotherapy: this is of special interest given the inefficacy of the current therapies. In particular, EATL type I, ENTL, t-MF and a subset of PTCL-NOS appear ideal candidates, whereas double staining on full sections for CD30 and for a nuclear B-cell marker (such as PAX5) are mandatory to precisely define CD30 expression in AITL. Interestingly, AITL cases of our series maintained higher percentages of positive cells than those previously reported, also with double immunostains (Table 1). However, it is crucial to underline that a standardized and reproducible CD30 search by immunohistochemistry is the prerequisite to precisely identify the most appropriate candidates for immune-therapy so as to ensure the best clinical results and the fewest drawbacks. In this regard, the choice of the most appropriate antigen retrieval method is mandatory for optimal immunohistochemical results: we regard a heat-based *plus* EDTA approach as the gold standard for CD30 assessment both in Hodgkin's and non-Hodgkin's lymphomas.[15]

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