Expression of CD52 in peripheral T-cell lymphoma

Peripheral T-cell lymphoma unspecified (PTCL/U) is a rare tumor characterized by poor treatment response and a dismal prognosis. We studied CD52 expression in 97 PTCL/U cases by immunohistochemistry on tissue-microarrays. Furthermore, CD52 gene expression was studied in 28 cases for which RNA was available. We found that CD52 is expressed in approximately 40% of PTCLs/U at the same level as in normal T-lymphocytes. Although other factors may play a role in the in vivo response to alemtuzumab, an anti-CD52 monoclonal antibody, the estimation of CD52 expression may provide a rationale for the selection of patients with a higher probability of treatment response.

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Peripheral T-cell lymphomas (PTCLs) are a heterogeneous group of tumors that the WHO classification basically subdivides into specified and unspecified (U).[1] In Western countries, they correspond to 15% of non-Hodgkin's lymphomas,[1] and more often present in advanced stage in middle aged/elderly patients who die rapidly despite aggressive therapies.[2] Recently, novel clinical/pathological scores have been proposed to improve prognostic stratification of PTCL/U patients.[3,4] However, novel targeted therapies are needed.

CDw52, originally characterized as a human leukocyte differentiation antigen, is present on the surface of most peripheral blood lymphocytes, macrophages, and monocytes at relatively high density. It is absent from myeloid cells, platelets, and erythroid cells as well as hematopoietic stem cells.[5] Campath-1H (alemtuzumab) is a humanized antibody against CD52 currently approved for chronic lymphocytic leukemia (CLL) therapy.[6] Furthermore, anti-CD52 showed interesting activity in T-prolymphocytic leukemia (disease characterized by high CD52 expression) and cutaneous T-cell lymphomas.[7] Only some data are available regarding alemtuzumab in PTCL/U,[7,8] which is often characterized by an aberrant lack of surface antigens.[4] Although other factors can affect the response to alemtuzumab *in vivo*, it is conceivable that lack of CD52 expression may play a major role in determining refractoriness to the compound. The aim of this study was to examine CD52 expression in PTCL/U to evaluate the potential for a more rationale use of alemtuzumab in this highly aggressive disorder.

We studied the expression of CD52 on tissue microarrays (TMAs) from 148 PTCL/U cases.[4] Ninety-seven out of 148 cases turned were evaluable due to core loss following repeated cutting. In addition, frozen material was available from 28 cases and gene expression profiles (GEP) were generated and compared to that of 20 samples of normal T-lymphocytes. These had been collected from peripheral blood and reactive tonsils of healthy donors.[9] In order to avoid the confusing effect of reactive components, only cases with 70-90% of neoplastic elements were chosen for GEP-analysis. GEP were generated and analyzed as previously reported[9] by using the HG-U133 2.0 plus micro-arrays (Affymetrix, Inc. *http://www.affymetrix.com/support/index.affx*). All patients had given permission for use of samples for research and study approval was obtained from the Local Ethical Committee.

Anti-CD52 (rat anti-human, monoclonal; Serotec ltd, Oxford, UK) was applied at a 1:200 dilution on a TechMate 500 immunostainer and revealed by the EnVision+ technique.[4] Before immunohistochemistry, the sections underwent antigen retrieval in citrate buffer (pH=6.0) in a micro-waver at 900W (3 cycles lasting 5' each). The TMAs were scored by two experienced pathologists, who estimated the number of positive cells. Cores were considered positive if 30% or more of the tumor cells were stained.[4]

We found that overall CD52 was down-regulated in PTCL/U when compared with normal T-lymphocytes (T-test, $p<0.0000001$). In particular, in 17/28 PTCL/U (60%), CD52 expression level was below the lowest value recorded in normal T-cells (Figure 1). In addition, it was detected by immunohistochemistry in 40 out of the 97 (41%) evaluable PTCLs. Notably, GEP paralleled CD52 staining in those cases undergoing both molecular analysis and immunohistochemistry. In particular, the median gene expression level was 5,031.00 compared with 1,804.00 in cases $CD52^+$ and $CD52^-$ at immunohistochemistry respectively ($p<0.05$). Interestingly, all the samples of normal T-lymphocytes, representing the supposed normal counterparts of this tumor, were CD52 positive, suggesting that PTCL/U often lacks its expres-

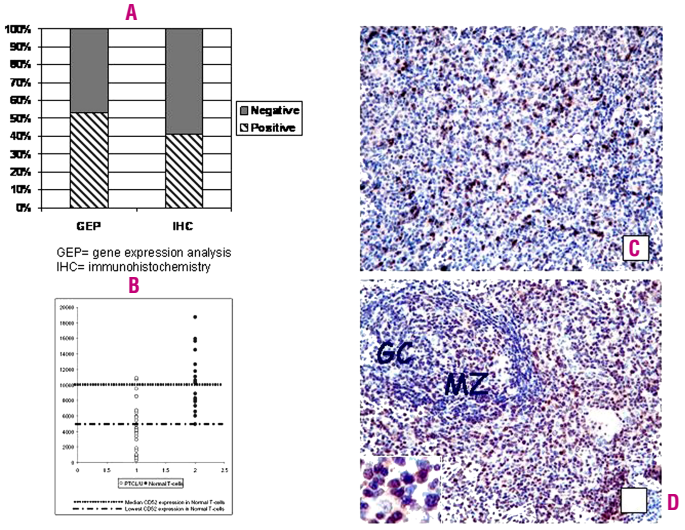

Figure 1. CD52 expression in PTCL/U. **A.** CD52 expression in peripheral T-cell lymphoma unspecified (PTCL/U) evaluated by gene expression analysis and immunohistochemistry as percentage of positive cases. **B.** CD52 gene expression in PTCL/U and normal T-cells. Raw expression data are reported on y axis. **C.** PTCL/U: neoplastic cells are negative at CD52 determination. Note the internal control represented by scattered reactive T-lymphocytes, which are clearly distinct from neoplastic elements due to both normal T-cell antigen expression and lack of cytological atipia. (Immunoperoxidase Envision$^+$ Technique; Gill's hematoxylin counterstaining; x200). **D.** PTCL/U: neoplastic cells infiltrating a normal germinal center (GC) strongly express CD52. Note the negativity of residual elements of the mantle zone (MZ) (Immunoperoxidase Envision+ Technique; Gill's hematoxylin counterstaining; x200). Inset: Neoplastic cells show *overt* nuclear shape and size variability and high nuclear/cytoplasmic ratio (Immunoperoxidase Envision$^+$ Technique; Gill's hematoxylin counterstaining; x600).

sion at both the RNA and protein level. This is not surprising as we found CD2, CD3, CD4, CD5, CD7, and CD8 variably expressed.[4] In other words, defectivity of T-cell associated antigens seems to be a hallmark of neoplastic transformation. It is of note, that our results, referring for the first time to paraffin-embedded cases, are in line with the those previously reported on frozen material.[10] Interestingly, our data seem to be in keeping with the clinical results obtained by Enblad *et al*.,[8] who found an overall response rate of 36% in PTCL treated with alemtuzumab.

Based on the above mentioned findings, the estimation of CD52 expression may provide a rationale for the selection of patients with a higher probability of responding to alemtuzumab by avoiding the risk of unwanted toxicity. Certainly, this implies standardization of the techniques adopted for CD52 evaluation. In our opinion, immunohistochemistry seems to represent an optimal approach. It can be applied to routine material in phase of other proteomic techniques, such as flow citometry or western blot. In fact, such techniques require fresh material that is only available in a small minority of lymphoma patients. This deserves further evaluation within prospective clinical trials.

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Addendum: after the acceptance of the present manuscript, similar immunohistochemical findings were reported by Rodig et al., cited in ref. #11.

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