Immunohistochemistry as a valuable tool to assess CD30 expression in peripheral T-cell lymphomas: high correlation with mRNA levels

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The extended use of brentuximab-vedotin was reported for CD30+ nonanaplastic peripheral T-cell lymphomas (PTCLs) with promising efficacy. CD30 status assessment is thus a critical factor for therapeutic decision, but the reliability of immunohistochemistry (IHC) in evaluating its expression remains to be defined. This prompted us to investigate the correlation between semiquantitative CD30 protein assessment by IHC and messenger RNA (mRNA) assessment by microarrays in a cohort of 376 noncutaneous PTCLs representative of the main entities. By IHC, CD30 expression was heterogeneous across and within entities and significantly associated with large tumor cell size. In addition to 100% anaplastic large-cell lymphomas, 57% of other PTCL entities were CD30-positive at a 5% threshold. CD30 protein expression was highly correlated to mRNA levels. mRNA levels were bimodal, separating high from low CD30-expressing PTCL cases. We conclude that IHC is a valuable tool in clinical practice to assess CD30 expression in PTCLs. (Blood. 2014;124(19):2983-2986)

Key Points

- IHC is a valuable clinical tool for assessing CD30+ PTCL patients who may respond to CD30-targeting treatment.
- CD30 mRNA and protein expression are highly correlated.

Introduction

CD30 is a transmembrane receptor with restricted expression on activated T and B cells in normal lymphoid tissues. In neoplastic conditions, strong CD30 expression is a feature of classical Hodgkin lymphoma and anaplastic large-cell lymphomas (ALCL). Given the high response rates seen in phase 2 studies, the novel CD30-targeting antibody drug–conjugated brentuximab vedotin (BV) was approved treating relapsed or refractory CD30+ HL and systemic ALCL.

PTCLs comprise a group of aggressive neoplasms characterized by unsatisfactory response to multiagent chemotherapy. Recent reports document promising efficacy of BV in nonanaplastic CD30+ PTCLs; however, CD30 protein expression in PTCLs is highly heterogeneous among entities, and the reliability of CD30 status determined by immunohistochemistry (IHC) in the context of prospective BV therapy remains a critical open question. This prompted us to investigate the correlation between CD30 protein expression as evaluated by IHC and messenger RNA (mRNA) expression in a large series of PTCLs to assess if the immunohistochemical scores sufficiently capture variations seen at the mRNA level. We show a consistently significant correlation between mRNA and protein expression for different PTCL entities, supporting that, in clinical practice, IHC is a valuable tool for determining CD30 status in PTCLs.

Study design

Patients and tumor samples

We selected a series of 376 PTCLs with formalin-fixed and frozen samples, diagnosed between 1999 and 2012, from the TENOMIC Consortium Biobank (approved by the local ethics committee, Comité de Protection des Personnes Ile de...
France 08-009; patients provided informed consent in accordance with the Declaration of Helsinki. In this framework, all cases were reviewed by a panel of expert hematopathologists and diagnosed according to the World Health Organization 2008 criteria. Of these, 238 cases had expression data (Affymetrix HG-U133 plus 2.0 chips, CD30 gene probe set: 206729_at), which include 54 cases from previously reported series.17-19

**CD30 expression assessment by combined IHC and gene expression profiling**

CD30 immunostains, performed on full sections as part of the diagnosis (by the submitting pathologists) or review process or for the purpose of this study (BerH2 antibody on the Ventana automated IHC platform, LYSA-Pathology Laboratory), were scored using a semiquantitative evaluation of the percentage of CD30 Immunohistochemistry scores on a 5-tiered scale (IHC scores: score 0, <5% of CD30 tumor cells; score 1, 5% to 24%; score 2, 25% to 49%; score 3, 50% to 75%; score 4, >75%; Figure 1A-F). To score CD30 expression in tumor cells only, CD30 immunostains were compared with the immunostains for CD3 and/or other relevant T-cell markers and CD20, especially in angioimmunoblastic T-cell lymphoma (AITL). CD30 staining intensity was also graded as weak, moderate, or strong. Cytological features, recorded as the proportion of small, medium, and large cells, were collapsed to 2 categories based on the predominance of the small or large cell component. Ten representative cases of AITL were subjected to double staining for the nuclear B-cell–associated marker PAX5 and CD30 (Figure 1G-H).

**Statistical analyses**

Spearman correlation coefficients were calculated between CD30 immunohistochemical scores and mRNA expression. Fisher’s exact test was used to evaluate the significance of cell size and staining intensity association with either CD30+ or CD30− samples. CD30 mRNA expression values were fitted with a 2-component Gaussian mixture model to test for bimodality (bimodality index threshold $\geq 1.1$), and the intersection of the curves was used as the mRNA threshold for CD30 positivity.20

**Results and discussion**

CD30 protein expression levels per entity are summarized in Table 1. Overall, 66% (248/376) of PTCLs expressed CD30 (score $\geq 1$), but only 34.5% (130/376) showed a high CD30 expression (score $\geq 3$).
Altogether, the CD30 immunohistochemical results presented in previous studies. 7-12,14-16,21,22 We found a higher percentage of CD30 immunohistochemical scores (CD30 staining intensities were significantly associated with higher immunohistochemical scores ($P = 5.68\times10^{-5}$) (supplemental Figure 2), or only AITL patients ($n = 64, p = 0.44, P = 3.17\times10^{-5}$) (not shown). However, a majority of cases ($n = 24/238, 10\%$), including 10 ALCs, 9 PTCL-NOSs, 4 AITLs, and 1 AITL, showed a discordant profile with a high CD30 IHC score ($\geq 3$) but low CD30 mRNA levels (Figure 1). These apparent discrepancies can be partly attributed to (1) PTCLs with a high proportion of CD30$^+$ tumor cells, but featuring weak staining intensity ($7/24$); (2) samples with a low tumor cell content ($2/24$); or (3) morphological variants of ALK$^+$ AITL (4/24). Conversely, all cases with high mRNA expression levels had detectable CD30 protein with an immunohistochemical score $\geq 2$. Altogether, these findings indicate that IHC is a sensitive method to assess CD30 expression.

Furthermore, CD30 mRNA expression was found to be bimodal and permits the identification of 2 groups of PTCLs: 1 group representing 66% of the cases expressing low levels of CD30 mRNA and a second group (34% of the cases) expressing high levels of CD30 mRNA corresponding to CD30 protein expression threshold between IHC scores 2 and 3 (Figure 1M). A bimodal distribution of CD30 mRNA expression levels was also observed when excluding the ALCs or when considering PTCL-NOS only (supplemental Figure 2), or only AITL patients (Figure 1M). The biological significance and therapeutic impact of CD30 bimodality warrant further studies.

Considering recent studies that show no apparent correlation between CD30 expression by tumor cells and BV response in lymphomas such as nonanaplastic PTCLs and diffuse large B-cell lymphomas as well as reported remissions in CD30-negative or weakly CD30-positive lymphomas, 6,23 the mechanisms determining BV antitumor activity remain unclear in these neoplasms. A possible hypothesis is that the drug diffuses from the targeted CD30$^+$ reactive cells to the tumor microenvironment and causes cytotoxicity on bystander tumor cells. 24 Given the presence of CD30$^+$ reactive B blasts in AITL, we reevaluated 24 of the CD30-negative AITL (score 0) by including CD30$^+$ B blasts, and 16 of them (66%) were reclassified as score 1. Further studies are needed to deepen the influence of combined CD30 expression levels from the tumor and its microenvironment in the therapeutic response to BV.

In conclusion, our study establishes for the first time a significant correlation between CD30 mRNA and protein expression, assessed by IHC, across different PTCL entities. We also show that IHC adequately captures CD30 mRNA expression features—namely

Table 1. CD30 immunohistochemical expression in PTCLs

<table>
<thead>
<tr>
<th>% of CD30$^+$ tumor cells</th>
<th>ALC ALK$^+$ (N = 61)</th>
<th>ALC ALK$^-$ (N = 19)</th>
<th>PTCL NOS (N = 141)</th>
<th>AITL (N = 97)</th>
<th>ENKTL (N = 28)</th>
<th>EATL (N = 14)</th>
<th>ATLL (N = 9)</th>
<th>HSTL (N = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score 0</td>
<td>0</td>
<td>0</td>
<td>59</td>
<td>36</td>
<td>15</td>
<td>7</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>$&lt;5%$</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>46</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Score 1</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5-24%</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Score 2</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>25-49%</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Score 3</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>50-75%</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Score 4</td>
<td>2</td>
<td>2</td>
<td>57</td>
<td>19</td>
<td>18</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>$&gt;75%$</td>
<td>2</td>
<td>2</td>
<td>57</td>
<td>19</td>
<td>18</td>
<td>0</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Total positive cases (scores 1-4)</td>
<td>61</td>
<td>19</td>
<td>82</td>
<td>61</td>
<td>13</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Strongly positive cases (scores 3-4)</td>
<td>58</td>
<td>19</td>
<td>63</td>
<td>63</td>
<td>46</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

ENKTL, extranodal natural killer/T-cell lymphoma; HSTL, hepatosplenic T-cell lymphoma.
heterogeneity and bimodality of CD30 expression in PTCLs. These findings indicate that IHC performed in a routine clinical setting is a valuable, practical tool to assess CD30 expression in PTCLs. Based on a large series of cases, we show that around 60% of AITL and PTCL-NOS patients and around 50% of patients with extranodal PTCLs have detectable CD30 expression using a 5% threshold. From a clinical perspective, this suggests that the majority of PTCLs may be potential candidates for CD30-targeting strategies, although further large clinical trials correlating response to BV and CD30 expression levels are needed to determine the criteria for patient eligibility.

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Authorship

Contribution: P.G. and L.d.L. designed and supervised the study; C.B., P.G., and L.d.L. reviewed all the cases for CD30 immunohistochemical expression; M.P.D. performed the statistical analysis; E.M. and M.D. supervised the statistical analysis; C.B., M.P.D., E.M., M.D., P.G., and L.d.L. analyzed and interpreted the results and wrote the paper; M.P., B.F., and A.M. participated in the pathologic review of patients entered in the TENOMIC consortium; C.H., R.D., and O.T. participated in documenting the patients; and all other authors contributed data and approved the paper.

Conflict-of-interest disclosure: C.B., C.H., R.D., and P.G. have acted on the advisory board for TAKEDA France. R.D. is a member of an independent data and safety monitoring committee for a clinical trial sponsored by TAKEDA. The remaining authors declare no competing financial interests.

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References

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