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## CD30 in Lymphoma: Its Role in Biology, Diagnostic Testing, and Targeted Therapy

### Discussants



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**Abstract:** CD30, a member of the tumor necrosis factor receptor superfamily, is a transmembrane glycoprotein receptor consisting of an extracellular domain, a transmembrane domain, and an intracellular domain. CD30 has emerged as an important molecule in the field of targeted therapy because its expression is generally restricted to specific disease types and states. The major cancers with elevated CD30 expression include Hodgkin lymphoma and anaplastic large T-cell lymphoma, and CD30 expression is considered essential to the differential diagnosis of these malignancies. Most commonly, CD30 expression is detected and performed by immunohistochemical staining of biopsy samples. Alternatively, flow cytometry analysis has also been developed for fresh tissue and cell aspiration specimens, including peripheral blood and bone marrow aspirate. Over the past several years, several therapeutic agents were developed to target CD30, with varying success in clinical trials. A major advance in the targeting of CD30 was seen with the development of the antibody-drug conjugate brentuximab vedotin, which consists of the naked anti-CD30 antibody SGN-30 conjugated to the synthetic antitubulin agent monomethyl auristatin E. In 2011, brentuximab vedotin was approved by the US Food and Drug Administration for use in Hodgkin lymphoma and anaplastic large cell lymphoma based on clinical trial data showing high response rates in these indications. Ongoing trials are examining brentuximab vedotin after autologous stem cell transplantation, as part of chemotherapy combination regimens, and in other CD30-expressing malignancies, including primary mediastinal large B-cell lymphomas, diffuse large B-cell lymphoma, lymphoma positive for Epstein-Barr virus, peripheral T-cell lymphoma not otherwise specified, and cutaneous anaplastic large cell lymphoma.

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# Biology and Expression of CD30

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## Biology of the CD30 Receptor

CD30 is one of several members of the tumor necrosis factor receptor (TNFR) superfamily.<sup>1</sup> Like many other members of the TNFR superfamily, CD30 is a transmembrane glycoprotein receptor consisting of an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain of CD30 contains 6 cysteine-rich stretches of amino acids, which help to form a scaffold of disulfide bonds, creating an elongated structure.<sup>2</sup> The intracellular cytoplasmic tail domain contains several serine/threonine residues that can become phosphorylated, creating docking sites for adaptor proteins to bind to and transduce signaling into the cell.<sup>3</sup> These adaptor proteins may include TNFR-associated factors (TRAFs) as well as a number of TRAF-binding proteins.<sup>4</sup>

Binding of the CD30 ligand (also called *CD153*) to CD30 induces trimerization and recruitment of molecules that result in signaling pathway activation.<sup>3</sup> These effects occur when ligand binding is transduced through the receptor to the intracellular portion of CD30, inducing TRAF binding (primarily TRAF2 and TRAF5) that then subsequently leads to the activation of signaling pathways.<sup>5,6</sup> For example, it has been demonstrated that binding of the CD30 ligand to CD30 induces changes in the subcellular localization and levels of TRAF2. Depending upon the particular cell conditions and signaling pathways induced, the CD30 receptor may trigger activation of the mitogen-activated protein kinase pathway or the inhibitor of  $\kappa$ B kinase/nuclear factor  $\kappa$ B (NF- $\kappa$ B) pathway.<sup>3,7,8</sup>

CD30 activation can lead to a series of pleiotropic effects resulting in proliferation, differentiation, or survival, depending upon the cell type, activation state, and transformation status, as well as the particular signaling pathway that is triggered.<sup>3</sup> The molecular mechanisms responsible for these differential effects are not completely understood, and the potential for situation-dependent and cell type-dependent pleiotropic effects is another characteristic that CD30 and its ligand share with other TNFR family members. Importantly, some of these differences may be mediated by the way in which the CD30 receptor becomes activated in various cells. For example, induction of CD30 signaling with an anti-CD30 antibody in an anaplastic large T-cell lymphoma (ALCL) cell line is associated with apoptosis, whereas the same antibody induces proliferation in Hodgkin lymphoma cell lines.<sup>9</sup> One important difference between these cells that may explain these opposing effects is the constitutive expression of NF- $\kappa$ B in the Hodgkin lymphoma cell lines.

The extracellular portion of CD30 is subject to enzymatic cleavage by metalloproteases, resulting in the release of 85-kDa soluble CD30 in the serum and/or bodily fluids.<sup>10</sup> Several studies have now demonstrated that levels of soluble CD30 correlate with disease activity in some cases of autoimmune disorders, and may be associated with the extent of tumor burden in some malignancies.<sup>11-14</sup> It is thought that high serum levels of soluble CD30 might provide an independent predictor of disease progression and poor prognosis in patients with CD30-positive lymphomas.

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## CD30 Expression in Normal and Pathologic Conditions

Overall, CD30 is expressed to only a limited degree in healthy tissue and cells.<sup>15,16</sup> The expression of CD30 during early human fetal development has been reported.<sup>17</sup> At between 8 and 10 weeks of fetal development, CD30 is expressed throughout fetal tissues derived from all 3 germ layers, including the gastrointestinal tract, the special glands of the postpharyngeal foregut, and the urinary, musculoskeletal, reproductive, nervous, and endocrine systems. By the 10th week of fetal development, CD30 expression can be found in the skin and the hematolymphoid system (particularly the thymus). After the 12th week of gestation, however, CD30 expression begins to become much more restricted, primarily to the hematolymphoid system. During this entire period of early fetal development, no CD30 expression can be observed in either the respiratory or cardiovascular systems. This strict regulation of CD30 expression during early fetal development suggests there are genetic or epigenetic mechanisms responsible for silencing expression of the gene encoding CD30.

In the healthy tissue of adults, minor CD30 expression can be observed in some resting cells and tissues, including cells in the lymph nodes, the tonsil, and the thymus, as well as in the decidual cells of the uterus and endometrial cells.<sup>3,18</sup> There is also some evidence of low-level CD30 expression in a small percentage of resting CD8-positive T cells.<sup>3,19</sup> The majority of CD30 expression in healthy adult tissues, however, is typically limited to activated B cells, T cells, and natural killer (NK) cells. The proportion of activated lymphocytes that are CD30-positive is less than 1% of the circulating cells in the blood.<sup>15</sup>

Viral infection can induce expression of CD30 on both T cells and B cells.<sup>16</sup> Indeed, the association of viral transformation with CD30 expression on peripheral blood lymphocytes resulted in an increased understanding of the role of CD30 as an activation molecule.<sup>18</sup> The percentage of CD30-positive activated peripheral blood cells can vary from fewer than 0.1% at baseline to as high as 95% by 3 days following a viral infection.<sup>16</sup> Several viruses have been implicated in inducing CD30 expression, including Epstein-Barr virus and human T-cell lymphotropic virus type I or II.<sup>15,20,21</sup> In CD4-positive T cells that are infected with the human immunodeficiency virus (HIV), activation of CD30 may provide a feedback mechanism supporting enhanced viral production, via TRAF2 induction of NF- $\kappa$ B.<sup>22</sup>

Soluble CD30 has been detected in the sera of patients infected with Epstein-Barr virus, hepatitis B virus, hepatitis C virus, and HIV.<sup>23-25</sup> Circulating levels of soluble CD30 may correlate with disease activity in HIV-infected patients,

with high levels considered to be an independent predictor of disease progression and poor prognosis.<sup>26</sup> The same has been suggested for patients with chronic hepatitis B infection.<sup>27,28</sup>

Several autoimmune and inflammatory diseases have also been found to be associated with highly elevated levels of CD30 expression, both on the surface of activated cells as well as in the soluble form of the molecule. High CD30 expression is particularly evident during periods of heightened disease activity or acute phases of the disease.<sup>12</sup> As with viral infections, high levels of soluble CD30 in the serum of these patients is suggestive of disease progression and poor prognosis.<sup>3</sup> Autoimmune and inflammatory diseases that have been found to exhibit elevated CD30 expression include rheumatoid arthritis, primary progressive multiple sclerosis, localized scleroderma, systemic lupus erythematosus, atopic dermatitis, asthma, and allergic rhinitis.<sup>3,29-31</sup> The association of CD30 with autoimmune and inflammatory diseases may be related to its high expression in T cells with a proinflammatory phenotype.

CD30 expression may also play a role in the development of graft-vs-host disease. Inhibition of CD30 is linked to a decreased incidence of graft-vs-host disease, an observation that has led to the exploration of the use of anti-CD30 antibodies as a means to prevent this condition.<sup>32</sup>

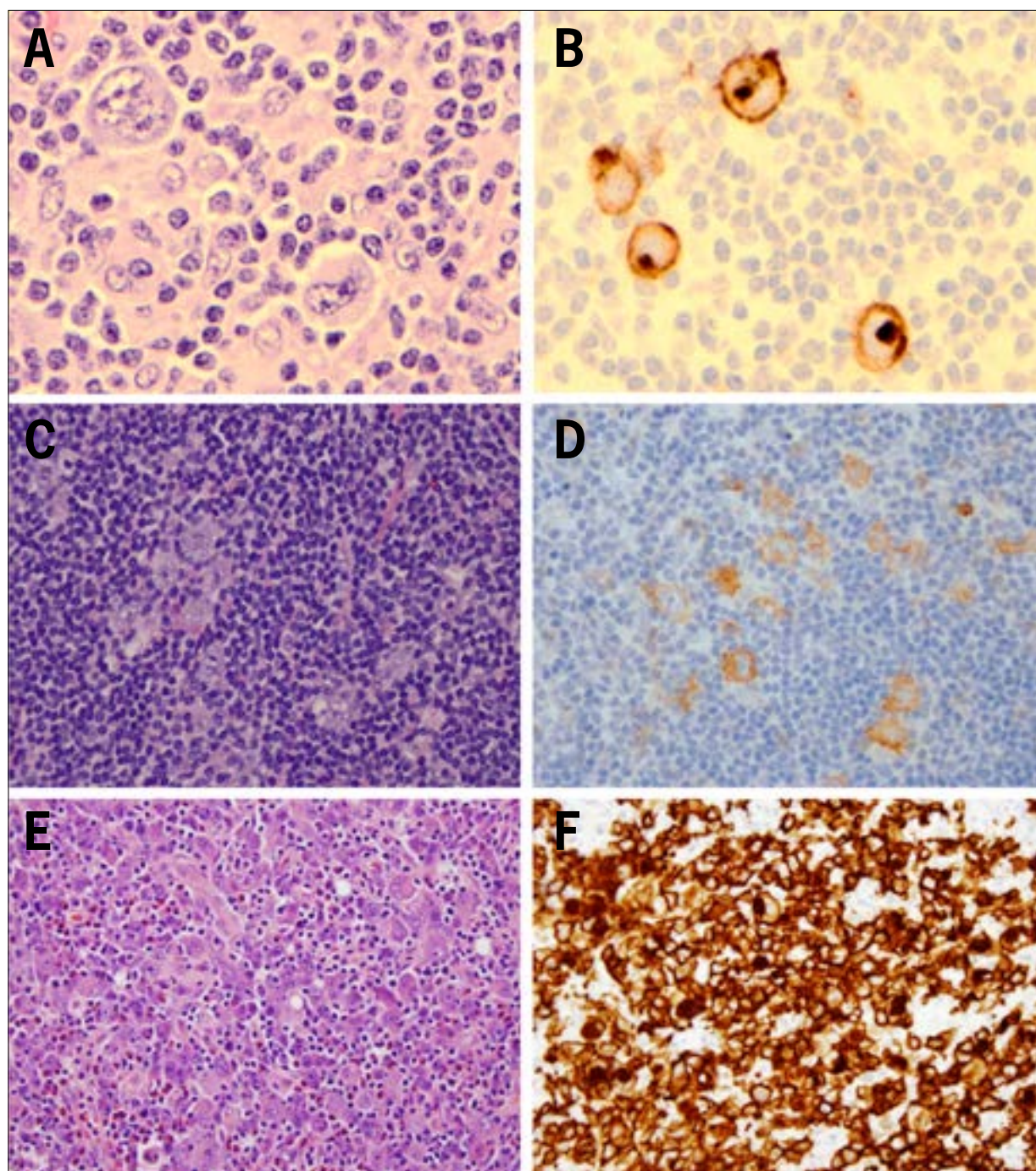
## CD30 Expression in Malignant Cells

By far, the most noted extent of elevated CD30 expression in diseased tissue is in malignancies. Chief among the malignancies that have elevated CD30 expression are Hodgkin lymphoma and ALCL (Figure 1). CD30 was first identified as an antigen specific to the Reed-Sternberg cells of Hodgkin lymphoma, a trait that was subsequently found to be shared by the large neoplastic cells of ALCL.<sup>15,33-35</sup> Later studies reported CD30 expression in more than 95% of cases of classical Hodgkin lymphoma.<sup>36</sup> By definition, all cases of ALCL are CD30-positive.<sup>37,38</sup>

In addition to the heightened expression of CD30 evident in Hodgkin lymphoma and ALCL, numerous reports have identified variable CD30 expression in other malignancies. However, it appears that in the vast majority of these cases, expression of the CD30 molecule occurs with far less frequency and/or at a lower level. For example, there is variable expression of CD30 in a variety of T-cell lymphomas; overall, CD30 expression is reported in approximately 30% of T-cell malignancies (Figure 2).<sup>39</sup> Approximately 32% to 52% of patients with peripheral T-cell lymphoma not otherwise specified show CD30 expression.<sup>40,41</sup> Enteropathy-associated T-cell lymphoma also shows relatively high CD30 expression.<sup>42,43</sup>

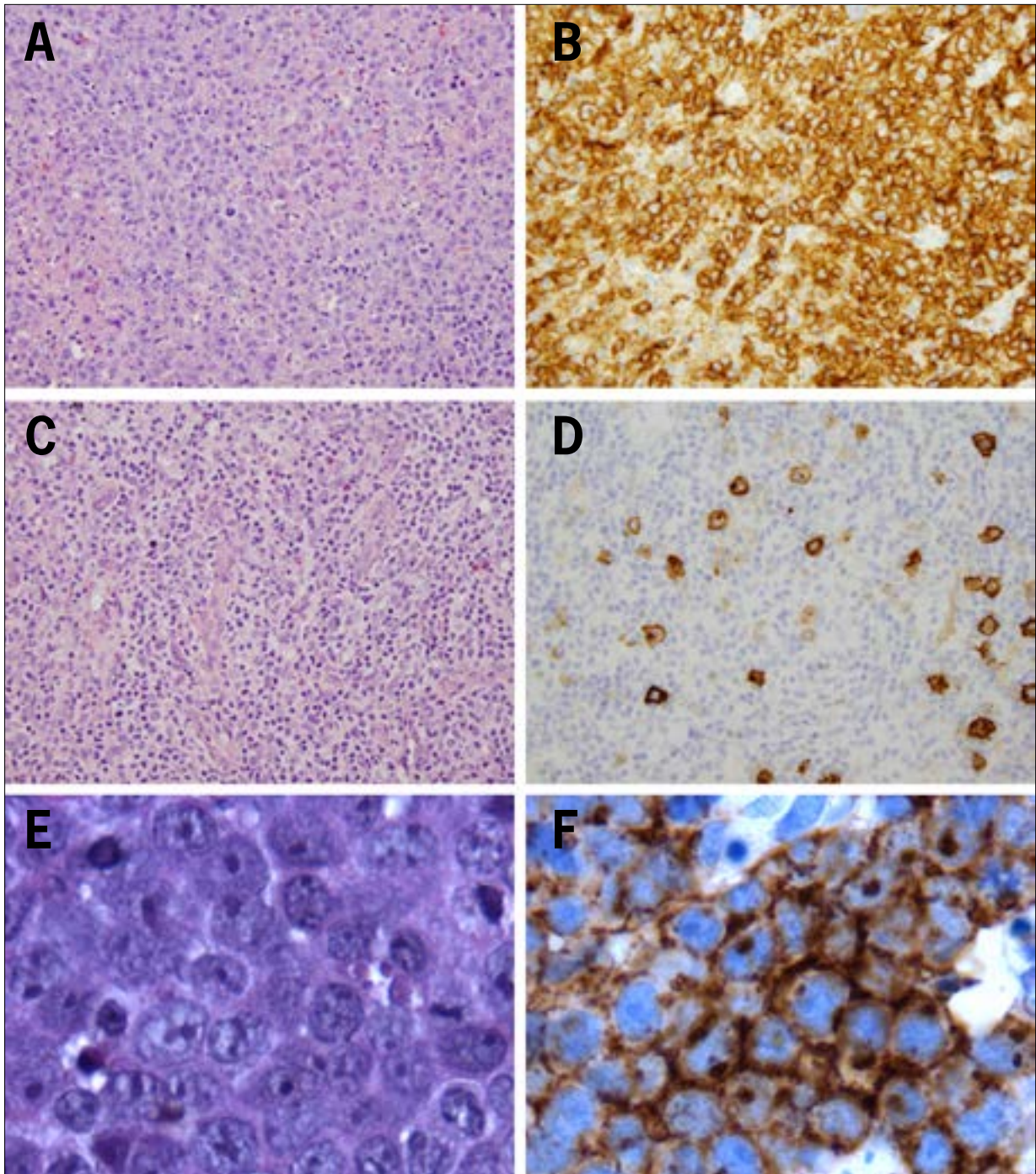
Overall, between 15% and 20% of B-cell malignancies are reported to be CD30-positive (Figure 2 [E and F]).<sup>44</sup> CD30 expression is reported in up to 26% of cases





**Figure 1.** **A.** Effacement of the lymph node in classical Hodgkin lymphoma (H&E, original magnification,  $\times 100$ ). **B.** The neoplastic cells show strong positive staining for CD30 (original magnification,  $\times 100$ ). **C.** Effacement of the lymph node in nodular lymphocyte predominant Hodgkin lymphoma (H&E, original magnification,  $\times 40$ ). **D.** The neoplastic cells show strong positive staining for CD30 (original magnification,  $\times 40$ ). **E.** Effacement of the lymph node in anaplastic large cell lymphoma (H&E, original magnification,  $\times 40$ ). **F.** The anaplastic large cell lymphoma cells show strong positive staining for CD30 (original magnification,  $\times 40$ ). H&E, hematoxylin and eosin stain. Courtesy: Dennis P. O'Malley, MD, and Ken H. Young, MD, PhD.





**Figure 2.** **A.** Effacement of the lymph node in peripheral T-cell lymphoma (H&E, original magnification,  $\times 40$ ). **B.** The T-cell lymphoma cells show strong positive staining for CD30 (original magnification,  $\times 40$ ). **C.** Effacement of the lymph node by angioimmunoblastic T-cell lymphoma (H&E, original magnification,  $\times 40$ ). **D.** CD30 stains show scattered immunoblasts, original magnification ( $\times 40$ ). **E.** Effacement of the lymph node by diffuse large B-cell lymphoma (H&E, original magnification,  $\times 100$ ). **F.** The diffuse large B-cell lymphoma cells show strong positive staining for CD30 (original magnification,  $\times 100$ ). H&E, hematoxylin and eosin stain. Courtesy: Roberto N. Miranda, MD, and Ken H. Young, MD, PhD.

of diffuse large B-cell lymphomas, although more recent reports suggest this rate may in fact be lower.<sup>45</sup> Importantly, among elderly patients with diffuse large B-cell lymphoma, CD30 expression is often associated with Epstein-Barr virus.<sup>46</sup> This correlation was shown in a Peruvian retrospective case review of elderly EBV-positive diffuse large B-cell lymphoma patients, which showed CD30 expression to be as high as 70% to 80%.<sup>47</sup>

CD30 is also expressed in solid tumors, although at a much lower frequency compared with hematopoietic-derived tumors. There is high expression of CD30 in testicular embryonal carcinoma and germ cell tumors.<sup>18,48,49</sup> In fact, CD30 is part of the diagnostic panel for testicular embryonal carcinoma. Other solid tumors in which CD30 has been reported with less frequency include ovarian cancer (5%), endometrial cancer (3%), and lung cancer (3%).<sup>50</sup> Interestingly, up to 14% of squamous cell skin carcinomas may have CD30 expression, as may approximately 10% of nasopharyngeal carcinomas.<sup>50</sup>

## Mechanisms Driving Increased CD30 Expression

Typically, naive T cells become activated in response to their first exposure to a cognate antigen. In *in vitro* laboratory experiments, activation can be achieved with a combination of anti-CD3 and anti-CD28 antibodies. CD30 becomes transiently expressed in these T cells subsequent to this primary activation. Expression of both interleukin 4 (IL-4) and CD28 are requisite for induction of CD30 expression.<sup>51</sup> This requirement for IL-4 may explain the high expression of CD30 in T-helper-2 cells, which rely upon IL-4 for induction. Upon second exposure to the cognate antigen, the expression of CD30 is more stable, suggesting that persistent expression of this molecule may be important for T-cell memory formation. This theory is supported by studies in CD30-deficient mice, which lack functional CD4 memory cells.<sup>52</sup> Of note, CD30 also promotes TH17 polarization.<sup>53</sup> This effect highlights the dichotomy ascribed to CD30, in that TH17 is proinflammatory whereas TH2-cells produce immunosuppressive cytokines.

At the molecular level, the promoter for the CD30 gene contains several binding sites for transcription factors such as Sp1 and STAT3.<sup>54</sup> In cell lines of both Hodgkin lymphoma and ALCL, CD30 expression has been shown to be regulated by the JunB transcription factor.<sup>55,56</sup> The CD30 gene also appears to be subject to epigenetic regulation, as its promoter includes regions known as CpG islands. In cell lines from Hodgkin lymphoma and ALCL, these CpG islands show distinct hypomethylation,<sup>57</sup> which results in increased expression of the CD30 transcript and subsequent higher levels of the CD30 protein. In contrast, certain tumors have

been shown to have hypermethylation of the CpG islands, with the subsequent silencing transcription of the CD30 gene. Importantly, this mechanism of gene silencing may be targeted for inhibition with hypomethylating agents, such as azacitidine or decitabine, or with pan-selective or isotype-selective histone deacetylase inhibitors.<sup>57</sup> Therefore, it is plausible that these epigenetic modifiers may provide a way to induce increased expression of CD30 in malignant cells that do not typically express this cell membrane protein. The use of a combination of epigenetic modifiers to increase CD30 expression could potentially broaden the use of CD30-targeted agents to include these cancers. Of note, one recent study used ascorbic acid to induce hypomethylation of the CD30 gene, resulting in increased expression of CD30.<sup>58</sup>

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# CD30 Diagnostics and Testing

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## Tools for Detection of CD30 Expression

CD30 is highly expressed by particular malignant cells but shows almost no expression by many normal cells. Therefore, CD30 expression can be used for diagnosis and as a highly specific target for treatment. There are several modalities available to assess for CD30 expression. The choice of modality is primarily dependent upon the sample type. In current histopathology practice, the most common sample type used for CD30 expression is investigation of formalin-fixed paraffin-embedded tumor tissue, although fresh frozen tissue may also be used in occasional scenarios. The quality of CD30 staining is often correlated with the quality of the biopsy specimen, regardless of whether it is a core needle biopsy, a fine needle aspiration, or an excisional biopsy. Overall, pathologists prefer to have a larger amount of biopsied tissue to allow for more accurate estimation of CD30 expression and intensity among tumor cells.<sup>1,2</sup>

CD30 expression is routinely detected by immunohistochemical staining in daily pathology practice. Recently, a flow cytometry approach was established for peripheral blood, bone marrow, and body fluid samples, as there are now anti-CD30 antibodies compatible with this modality. An enzyme-linked immunosorbent assay (ELISA) is also useful for measuring levels of soluble CD30 expression. A comparison of immunohistochemistry with flow cytometry showed that these 2 methods were well correlated for detection of CD30 expression on lymphoma and leukemia cells.<sup>3,4</sup> Table 1 summarizes the utility, pros, and cons for these CD30 detection methods. Immunohistochemistry combines the advantage of antigen expression with tumor cell morphologic features, facilitating diagnosis and classification. It is the method most commonly used by practicing pathologists and physicians. However, the quality of immunohistochemical staining closely relies on the tissue handling and processing methods. Interpretation of the results is significantly impacted by the tissue fixation process, including the fixation duration, type of fixatives, antigen retrieval, antibody staining procedure, and quality of tissue, as well as the presence of any crush artifacts or necrotic changes. Further improvement of staining standardization will allow immunohistochemistry to be optimized for pathology practice.

Flow cytometry has become an increasingly powerful approach to CD30 detection in clinical use, particularly in hematologic malignancies, because blood and bone marrow specimens are easily obtainable. In solid tissue biopsies, the use of flow cytometry is determined by the availability of fresh tissue. Availability of a small amount of biopsy tissue will prioritize routine histopathology evaluation over flow cytometry analysis. Therefore, in many clinical scenarios, flow cytometry is selected when the available specimens consist of blood, bone marrow, or body fluids. In addition to its high efficiency and better turnaround time, flow cytometry also offers the advantages of better standardization and high sensitivity to identify patients with weak CD30 expression cells. Current multicolor flow cytometry technology provides a powerful method for antigen profiling analysis in both clinical and research settings.

There are currently 5 different types of anti-CD30 monoclonal antibodies used routinely for CD30 diagnostics. They are designated Ki-1, Ber-H2, Ber-H4, Ber-H6, and Ber-H8. The Ber-H2 antibody is the most compatible with fresh frozen tissue, and all 5 of these antibodies work well with formalin-fixed paraffin-embedded tissue. In the United States, Ki-1 and Ber-H2 are the most widely used for immunohistochemistry; these 2 antibodies recognize different epitopes of the CD30 extracellular domain. With immunohistochemistry, the typical CD30 staining pattern is represented by strong staining of the cell membrane coupled with weak staining in the cytoplasm. Cytoplasmic staining is likely caused by recognition of a CD30 precursor protein in the Golgi apparatus.

As was previously mentioned, membrane-bound CD30 may be cleaved to produce a soluble form of the molecule. Increased serum levels of soluble CD30 have been observed in a variety of pathologic conditions. There is some evidence that high soluble CD30 levels correlate with tumor burden, poor prognosis, and disease activity, and therefore CD30 may have some prognostic significance in certain cancers, such as Hodgkin lymphoma and ALCL. In addition, soluble CD30 has been identified in a variety of autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, atopic dermatitis, Graves disease, Hashimoto thyroiditis, Wegener's granulomatosis, and Omenn syndrome.

**Table 1.** Pathologic Tests for Assessment of CD30 Expression

Test	Utility	Pro	Con
IHC	Most frequently used for diagnosis in clinical practice Uses FFPET and frozen tissue section	Supports diagnosis effectively by combining biomarker expression and morphology Standardized staining procedure Uses appropriate controls (tonsil, reactive lymph node) Antigen retrieval method (HIER plus EDTA) established as gold standard Membrane and cytoplasmic staining pattern Percentage and intensity calculation for quantitation of CD30 expression Cost-effective	Results influenced by the quality of the biopsy tissue: size, necrosis, fixation, reagents, and processing Interpretation influenced by staining method Antigen retrieval may not be effective for all biomarkers False-positive and -negative results Semiquantitative measurement 1-2 days turnaround time
Flow Cytometry	Increasingly used for diagnosis in clinical practice Uses fresh cells and frozen tissue, especially for blood, bone marrow, and body fluids	Standardized operation Shows antibody reaction pattern (dim, bright, moderate, cell population) and supports detection of weakly expressed cells Uses appropriate reagents and instrument-quality controls Quantitation of CD30 expression profile, distribution, and localization in specific cell populations Powerful method for blood, bone marrow, and body fluid specimens Multicolor analysis with other biomarkers facilitate phenotypic profiling Quick turnaround time of 3 hours	Viable cells and tissue only, such as blood, bone marrow, and body fluids Requires more tissue than the amount usually obtained with a small needle biopsy Relatively expensive
ELISA	Research	Soluble form of CD30 in the blood and body fluids Quantitative measurement Quick turnaround time of 3 hours Highly efficient, dynamic, and can be performed for many specimens simultaneously	Plasma, serum, and body fluid specimens Biologic correlation is unclear

EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FFPET, formalin-fixed paraffin embedded tissue; HIER, heat-induced epitope retrieval; IHC, immunohistochemistry.

In routine pathology practice, the immunohistochemistry method provides sufficient, reliable support during the diagnostic workup, and it is more cost-effective compared with flow cytometry. The ELISA method has been used to detect soluble CD30, which is increased in patients with cancer and autoimmune disorders. The results of ELISA, however, can be unreliable, and its use as a diagnostic and prognostic biomarker requires further investigation.

### ***Challenges in Quality Assurance***

There are several ways to ensure for quality control in CD30 testing, regardless of whether immunohistochemical staining or flow cytometry is used. The most critical of these measures involves the handling of the tissue samples. For immunohistochemistry, reliable positive and negative controls are required, as is the use of an optimal antigen retrieval method. In flow cytometry, the gating strategy and cell population are critical. Other important factors include preanalytic components, such as use of the appropriate reagents, instrument equilibrium and calibration using ink-staining procedures, and the skills of the technician.

### **Challenges for CD30 Expression Testing**

By far the most critical challenge for obtaining the correct results by CD30 testing comes from the biopsy sample itself, including the type of the tissue used, the size of the sample, whether necrosis is present, and the fixation method used.<sup>5</sup> Staining artifacts can occur if fixation is delayed, the fixation volume or time are insufficient, or the tissue block is too large. Another challenge associated with CD30 immunohistochemical staining is determining the type of CD30 antibody to be used and the specific staining procedure to be followed. It is important to choose the appropriate controls and antigen retrieval method. Additionally, a determination should be made a priori regarding how the CD30 expression pattern will be quantitated, including whether it will be reported as a percentage (of what cells) and/or as an intensity of expression among tumor cells.

When flow cytometry is chosen as the method of choice for CD30 expression testing, parameters such as the gating strategy and cell population to be analyzed must be well selected. There is also a need to know the antibody reactivity pattern expected in selected normal lymphoid cells and background non-neoplastic cells.

**Table 2.** Common Immunophenotypic Markers in Lymphoma Diagnosis

Lymphoma	Common Markers
Hodgkin lymphoma	CD3, CD15, CD20, CD30, CD45, CD79a, PAX5, OCT2, BOB1, EMA, EBV (LMP1)
ALCL, ALK-positive	CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD30, ALK, clusterin, EMA
ALCL, ALK-negative	CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD25, CD30, CD43, CD56, CD68, ALK, clusterin, EMA
PTCL	CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD25, CD30, CD43, CD56, CD68, ALK, BCL6, clusterin, CXCR-13, granzyme B, PD-1, perforin, TIA-1, TCR- $\alpha/\beta$ , TCR- $\delta$
PTCL-NOS	CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD25, CD30, CD43, CD56, CD68, ALK, BCL6, clusterin, CXCR-13, granzyme B, PD-1, perforin, TIA-1, TCR- $\alpha/\beta$ , TCR- $\delta$

ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase; PTCL, peripheral T-cell lymphoma; NOS, not otherwise specified; TCR, T-cell receptor.

### When CD30 Testing Should Be Performed

A number of hematologic malignancies have been identified in which CD30 expression testing may prove useful for diagnosis, differential diagnosis, and risk stratification.<sup>6</sup> Hodgkin lymphoma and ALCL are the 2 forms of lymphoma in which CD30 expression is present in nearly all patients. CD30 expression is seen in 98% of patients with classical Hodgkin lymphoma.<sup>7</sup> CD30 expression is considered to be diagnostically essential for ALCL. CD30 staining constitutes an essential part of the differential diagnostic workup for both Hodgkin lymphoma and ALCL. For example, Hodgkin lymphoma is CD30-positive and CD15-positive, but negative for LCA, CD3, TIA-1, and EMA. In contrast, ALCL is positive for CD30 and negative for CD15, but positive for LCA, pan T-cell antigens (variable), TIA-1, and EMA; it is also positive for anaplastic lymphoma kinase (ALK) in some cases. When used together, these biomarkers can provide information for an accurate differential diagnosis of these lymphoid malignancies in daily pathology practice. Several studies have shown that agreement among expert pathologists is relatively poor for many lymphoma subtypes.<sup>8,9</sup> A study from the International T-Cell Lymphoma Project found that 10% to 20% of diagnoses were incorrect owing to the lack of specific diagnostic biomarkers.<sup>8</sup> In the setting of ALK-positive ALCL, NK-cell lymphoma, and adult T-cell leukemia, accuracy and reliability are relatively higher owing to the presence of unique immunohistochemical biomarkers. It is suggested that expert pathology review to reach diagnostic consensus should be considered the standard of care.<sup>8,9</sup> In addition, better and more specific diagnostic biomarkers are urgently required for use in daily practice to improve the accuracy and reproducibility of diagnosis in some difficult lymphoma subtypes. For example, new immunostains for CD30, ALK, CXCR-13, and PD-1 should improve the diagnostic accuracy for Hodgkin lymphoma, ALK-positive ALCL, and angioimmunoblastic T-cell lymphoma. Table 2 shows the common immunophenotypic biomarkers used in the diagnosis of these lymphoma subtypes.

CD30 may aid in the risk stratification and differential diagnosis of diffuse large B-cell lymphoma and primary mediastinal large B-cell lymphoma. Among T-cell lymphomas, diagnosis of several subtypes may benefit from additional CD30 testing, such as NK/T-cell lymphoma and ALCL. CD30 expression is important for diagnosis of cutaneous T-cell lymphomas, including primary cutaneous ALCL, lymphomatoid papulosis, and mycosis fungoides (especially when transformed). CD30 may also aid in the recognition of posttransplant lymphoproliferative disorder and lymphoblastic leukemia/lymphoma (both B-cell and T-cell types) in occasional scenarios.

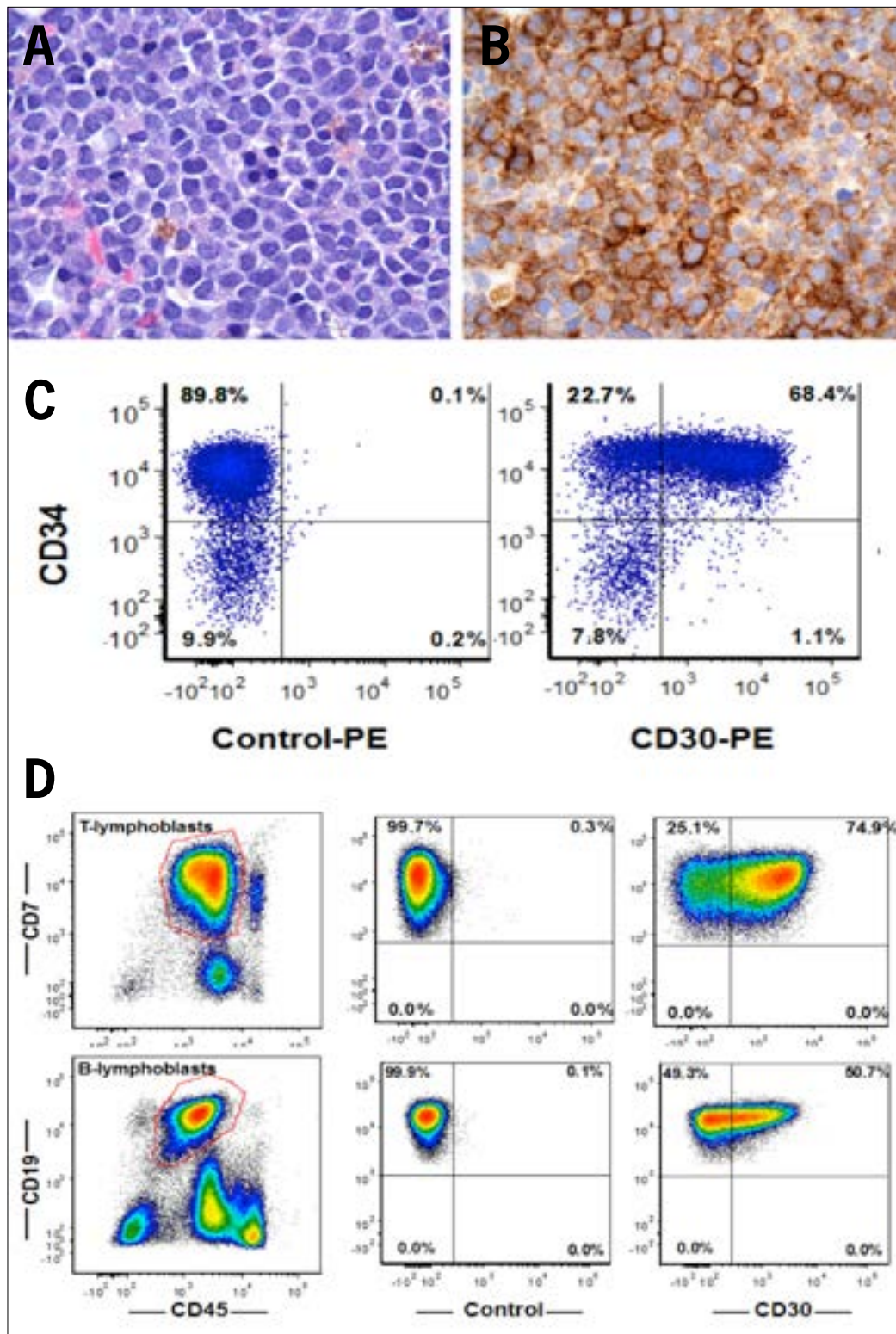
Recently, CD30 expression was identified in a subset of patients with acute lymphoblastic leukemia, acute myeloid leukemia (Figure 3), and myelodysplastic syndrome.<sup>3,4</sup> Mast cell diseases and Castleman disease may represent occasional instances in which CD30 expression could be useful in recognition of the disease phenotype, although this use has been shown in few patients. In addition to hematologic malignancies, several solid tumors, including seminoma, embryonal carcinoma, endometrial carcinoma, germ cell tumors, and melanoma, can also show variable CD30 expression. The biology and role of CD30 in the development and progression of these malignancies are unknown.

### Recommendations for CD30 Expression Testing

Guidelines regarding CD30 staining are based on those from the National Comprehensive Cancer Network commonly used for Hodgkin lymphoma and ALCL in the presence of appropriate controlled working procedures.<sup>10</sup> A membrane and dot-like cytoplasmic staining pattern for CD30 is typical of these diseases, whereas strong cytoplasmic staining is seen in plasma cell neoplasms and membranous staining in embryonal carcinoma. There is usually little to no background staining.

The anti-CD30 antibodies that have been proposed for diagnostic use (Ber-H2, 1G12, JCM182, 15B3, and CON6D/B5) commonly achieve good staining quality





**Figure 3.** A. Acute myeloid leukemia (H&E, original magnification  $\times 100$ ). B. The acute myeloid leukemia cells show strong positive staining for CD30 (original magnification  $\times 100$ ). C. Flow cytometric immunophenotyping of CD30 expression in gated CD34-positive myeloid blast population in comparison to the control. D. Flow cytometric immunophenotyping of CD30 expression in gated CD7-positive and CD19-positive T-lymphoblast and B-lymphoblast populations in comparison to the control in corresponding T-lymphoblastic and B-lymphoblastic leukemia patients. Courtesy: Sa A. Wang, MD, and Ken H. Young, MD, PhD.

and accuracy. The antigen retrieval method used with these antibodies is heat-induced antigen retrieval. Tonsil tissue is recommended as the positive control, although activated T cells and B cells from the interfollicular and perfollicular regions may also work well as internal controls. Quantitation of CD30 expression is calculated as either a percentage or an intensity of staining among the cells of interest. The methods for tissue handling and staining greatly impact the interpretation of immunohistochemical results.

The Nordic Immunohistochemical Quality Control (NordiQC) group promotes the quality of immunohistochemistry and provides information on its clinical use.<sup>11</sup> Since 2004, the number of laboratories that have participated in CD30 expression testing with the NordiQC has more than doubled, from 74 to 172. However, the proportion of these laboratories that can provide optimal staining has decreased from 92% to 77% during the same time.<sup>11</sup> The major reasons for inadequate staining in these laboratories include the use of antibodies at an incorrect concentration (either too high or too low), inadequate time for antigen retrieval, inadequate washing to remove unbound antibody, and poor specimen handling during the fixation and sectioning steps.

The National Institute for Health and Care Excellence (NICE) has also developed guidelines for several clinical and diagnostic assessment programs.<sup>12</sup> Staining for CD30 is included as a pilot program. This program relies on external healthcare experts to develop protocols. Thus far, it has not yet produced CD30-specific standard protocols.

In our recent study of patients with diffuse large B-cell lymphoma, 20% expression was used as the threshold for CD30-positive status.<sup>13</sup> Therefore, any diffuse large B-cell lymphoma patients with less than 20% expression in the tumor cells were considered negative. Some studies have used 30% expression as a cutoff, but it is likely that this level provides very similar results to those obtained with a cutoff of 20%.

Including CD30 staining in the laboratory workup can increase the reproducibility of the diagnosis, especially in cases of lymphoma. For both Hodgkin lymphoma and ALCL, CD30 staining should be performed in all patients. In many cases, CD30 expression staining may help classify a particular cancer subtype for better risk stratification and support of clinical trials.

### Impact of CD30 Expression Testing for Understanding Lymphoma

Two studies were published in 2013 that demonstrated the potential importance of CD30 expression testing in determining an optimal treatment strategy. The first study evaluated the expression of CD30 among a variety of peripheral T-cell lymphomas other than ALCL.<sup>14</sup>

Overall, 43.2% of cases were found to be CD30-positive, with a score of 2+ or more.

In the second of these studies, gene expression profiling and immunohistochemistry were performed on peripheral T-cell lymphoma samples.<sup>15</sup> When CD30-positive peripheral T-cell lymphoma was compared with CD30-negative cases, there were unique molecular and phenotypical signatures involving T-cell receptor pathways that were significantly downregulated in the CD30-positive cases. In patients with CD30 expression, the tyrosine kinases involved in proximal T-cell receptor signaling, T-cell differentiation, and activation (Lck, Fyn, and Itk) were either absent or found at levels that were significantly decreased as compared with CD30-negative patients. The cell surface antigens responsible for T-cell differentiation and activation, such as CD69, ICOS, and CD52, were also markedly decreased in expression in CD30-positive peripheral T-cell lymphoma. In contrast, several transcription factors, including JunB, MUM1/IRF4, STAT3, C/EBP $\beta$ , and cyclin D3, were significantly upregulated. The differential gene expression signatures may partially explain the better prognosis of CD30-positive peripheral T-cell lymphoma as compared with CD30-negative peripheral T-cell lymphoma.

It is known that diffuse large B-cell lymphoma has multiple types of variants, subgroups, subtypes, and other entities. The most common morphologic variants include centroblastic, immunoblastic, and anaplastic. Each of the different variants of diffuse large B-cell lymphoma is associated with a particular outcome. It is possible that CD30 may contribute to the prognosis and pathogenesis of these patients. There are 3 major molecular subtypes of diffuse large B-cell lymphoma. In cases of the germinal center B-cell-like variant, CD30 expression correlates with CD10 and other germinal center biomarker expression. In contrast, in cases of the activated B-cell-like variant, CD30 expression instead correlates with MUM-1 and FOXP-1 expression.<sup>13</sup> CD30 is expressed at a very high frequency in patients with Epstein-Barr virus.<sup>16,17</sup> Intrasinusoidal distribution of CD30-expressing cells can mimic ALCL presentation.

In diffuse large B-cell lymphoma, CD30 expression was found to correlate with patients' treatment response and survival.<sup>13</sup> As compared with CD30-negative disease, CD30-positive disease was associated with a significantly higher overall survival ( $P=.0327$ ) and an improved progression-free survival that nearly reached significance ( $P=.0544$ ). Gene expression profiling found 99 genes that were differentially upregulated and 55 genes that were downregulated in CD30-positive disease. Upregulations mainly included genes involved in NF- $\kappa$ B regulation, lymphocyte proliferation, and death receptors. Downregulations primarily involved B-cell receptor pathways, which are known to be important in the pathogenesis of this tumor type, as well as cytokine and chemokine pathways.

**Table 3.** Benefits of CD30 Biomarker Testing

Unique cell surface biomarker for diagnosis and differential diagnosis in Hodgkin lymphoma, non-Hodgkin lymphoma, and solid tumors
Critical cell surface biomarker that provides a therapeutic target for immunotherapy
Correlation of its expression with CD30 downstream signaling pathway genes and biomarkers that are related to lymphoma pathogenesis
Interpretation of expression and biologic significance is beneficial to a better understanding of CD30-positive lymphoma

When diffuse large B-cell lymphoma patients with CD30-positive disease are compared with those who are CD30-negative, 3 biologic parameters appear to show a significant correlation with CD30 status. In patients who are CD30-positive, coexpression of Myc/Bcl-2 is significantly lower (23% vs 36%;  $P=.0483$ ), chromosomal aberrations in Myc are significantly lower (0% vs 13%;  $P=.0100$ ), and p21 expression is significantly higher (24% vs 11%;  $P=.0034$ ).<sup>13</sup> Without these factors, CD30 is not an independent prognostic factor for better survival in diffuse large B-cell lymphoma patients.

Infection with the Epstein-Barr virus was evaluated independently in diffuse large B-cell lymphoma patients. Patients with Epstein-Barr virus and CD30-positive disease had a significantly lower overall survival compared with patients with CD30-negative disease (regardless of whether the CD30-negative patients were positive or negative for Epstein-Barr virus).<sup>16</sup> Gene expression analysis demonstrates that NF- $\kappa$ B activation and the JAK-STAT pathways are critical.

CD30 expression as assessed by immunohistochemistry and flow cytometry has been recently used to further refine the diagnosis, differential diagnosis, and biologic characterization of lymphoma subtypes. Quantitative analysis of CD30 expression and intensity provides valuable information for risk stratification in lymphoma patients, thereby rendering rationale for effective targeted therapy. As CD30 has been expressed in a subset of high-grade T-cell, B-cell, NK-cell and Hodgkin lymphoma patients, it is recommended that immunohistochemistry and/or flow cytometry be used in all high-grade lymphoma patients during the diagnostic workup. This approach may also apply to solid tumors that express CD30 to variable degrees, such as seminoma, melanoma, endometrial carcinoma, and germ cell tumors. Accurate and reliable CD30 detection and measurement, for both neoplastic and non-neoplastic disease, likely provide information that is valuable to treatment decisions and regimen selection, considering the recent advancements in the development of therapies targeting the CD30 antigen. Standardization of detection methods; appropriate tissue handling and processing; quality control and assurance; well-controlled staining systems,

**Table 4.** Components of Optimal Testing for CD30 Expression

Well-controlled tissue handling, fixation, and processing
Adequate antigen retrieval method and procedure, including HIER plus EDTA, to achieve optimal immunostaining results
Quality control and quality assurance of techniques used in the immunostaining procedures
Appropriate positive (tonsil and lymph node) and negative controls
Standardization of the immunostaining detection method, reagents, and operation protocol
Consistent image capture for quantitation of expression percentage and intensity

HIER, heat-induced epitope retrieval; EDTA, ethylenediaminetetraacetic acid.

reagents, or commercial kits; reproducible techniques; and development of reference methods with consensus guidelines should be integrated into daily pathology practice in community settings and academic hospitals to support personalized therapy in the era of molecular medicine.

## Summary

Optimal quality assurance control and protocol standardization for CD30 staining will be critical for improving testing accuracy and reproducibility of testing (Table 3). Proper sample handling and antigen retrieval are critical, and the detection method used requires standardization (Table 4). Current lymphoma classification integrates a number of immunophenotypic and genomic biomarkers. The addition of CD30 and other biomarkers, such as Bcl-2, Myc, and p53, may offer a better understanding of lymphoma pathogenesis and disease progression, as well as provide critical support for risk stratification and recommendations for treatment regimens. Incorporation of correlative studies with CD30 expression into clinical trials will not only drive a better understanding of the pathophysiology of CD30-positive diseases and the downstream signaling pathway, but also introduce important therapeutic concepts and targets for cancer patients in the near future.

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# Evolution of CD30 as a Target in Hodgkin Lymphoma, Mature T-Cell Lymphoma, and Other Lymphomas

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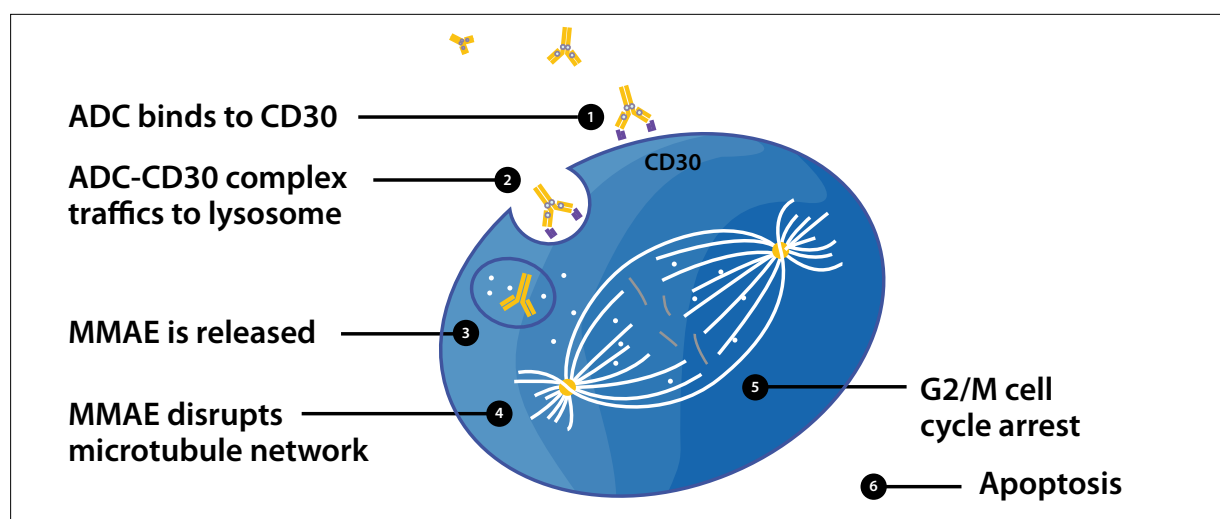
## Targeting CD30

CD30 has emerged as an important molecule in the field of targeted therapy because its expression is generally restricted to certain disease states, which makes it an ideal target for inhibition. Early efforts to target CD30 with the use of naked antibodies met with variable success.<sup>1</sup> The fully humanized anti-CD30 monoclonal antibody iratumumab (MDX-060) had only modest activity in a phase 1 trial of patients with relapsed or refractory CD30-expressing Hodgkin lymphoma or ALCL.<sup>2</sup> Similarly, in phase 1 and 2 trials, the chimeric anti-CD30 monoclonal antibody SGN-30 produced few responses in patients with ALCL, and no responses among Hodgkin lymphoma patients.<sup>3,4</sup>

Development of the novel antibody-drug conjugate brentuximab vedotin (SGN-35) was a major advance in the attempt to clinically target CD30. This agent consists of the previously investigated naked anti-CD30 antibody SGN-30 conjugated to the synthetic antitubulin agent monomethyl auristatin E (MMAE; Figure 4). The 2 moi-

eties in brentuximab vedotin are conjugated via a dipeptide linker that is cleaved by lysosomal enzymes upon receptor-mediated internalization into target cells.<sup>5-7</sup> Once released, MMAE induces cell growth arrest followed by apoptosis.<sup>5,8</sup> Additionally, accumulation of MMAE in the extracellular space may add to its potent anticancer activity, whereby it can exert its cytotoxic effects on neighboring tumor cells because it is itself membrane permeable.<sup>9</sup> Another potential mechanism of action attributed to brentuximab vedotin is related to the CD30-directed antibody, which may interrupt binding of the CD30 ligand to its receptor.<sup>5</sup>

Preclinical experiments showed that the addition of the cytotoxic MMAE molecule to the anti-CD30 antibody resulted in significantly enhanced growth arrest of CD30-expressing cell lines and greater antitumor activity in mouse xenograft models of both Hodgkin disease and ALCL.<sup>8</sup> This activity was highly specific, with the antibody-drug conjugate associated with a 300-fold greater activity in CD30-expressing cells as compared with CD30-negative cells. Early clinical studies of brentuximab vedotin confirmed its prom-



**Figure 4.** Brentuximab vedotin is an antibody-drug conjugate (ADC) that targets CD30. It consists of the naked anti-CD30 antibody SGN-30 conjugated to the synthetic antitubulin agent monomethyl auristatin E (MMAE).

using preclinical activity, resulting in significant and clinically meaningful responses in both Hodgkin disease and ALCL.

### Clinical Development of Brentuximab Vedotin in Hodgkin Lymphoma and ALCL

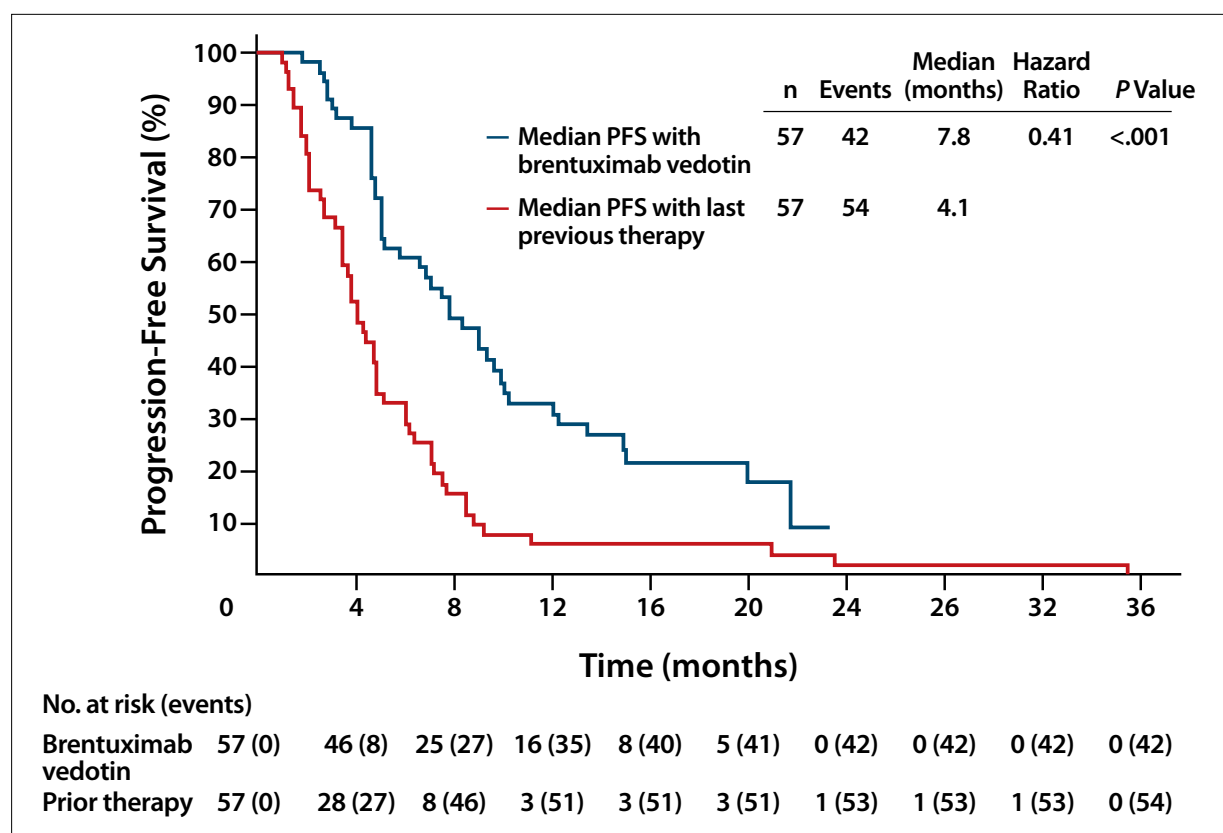
In a pilot, open-label, multicenter, dose-escalation, phase 1 trial, single-agent brentuximab vedotin was investigated in 45 patients with relapsed or refractory CD30-expressing lymphomas (42 patients with Hodgkin lymphoma, 2 patients with systemic ALCL, and 1 patient with angioimmunoblastic T-cell lymphoma).<sup>10</sup> These patients were heavily pretreated (median of 3 prior chemotherapy regimens), and most (73%) had undergone a previous stem cell transplantation. Brentuximab vedotin was administered on a schedule of once every 3 weeks, at doses ranging from 0.1 to 3.6 mg/kg. The maximum tolerated dose was identified as 1.8 mg/kg, and the associated toxicities were primarily grade 1 or 2 and included fatigue (36%), pyrexia (33%), diarrhea (22%), nausea (22%), neutropenia (22%), and peripheral neuropathy (22%). The objective response rate in the overall treatment population was 38% (with 24% complete remissions), but increased to 50% among patients who received the maximum tolerated dose. Computed tomography imaging confirmed tumor regression in 86% of patients. Responses proved to be durable, with a median duration of 9.7 months.

A subsequent phase 1 dose-escalation study was also performed, in which single-agent brentuximab vedotin was administered to 44 patients with relapsed or refractory CD30-expressing lymphomas (38 patients with Hodgkin lymphoma, 5 patients with systemic ALCL, and 1 patient with peripheral T-cell lymphoma not otherwise specified).<sup>11</sup> Again, patients were heavily pretreated (median of 3 prior chemotherapy regimens), and 68% had undergone prior stem cell transplanta-

tion. Unlike the previous phase 1 trial, which administered the agent once every 3 weeks, this study administered brentuximab vedotin once weekly for 3 weeks of a 4-week cycle. Doses ranged from 0.4 to 1.4 mg/kg, and the maximum tolerated dose with this schedule was found to be 1.2 mg/kg. Although the toxicities reported in the study were similar to those in the pilot trial, their incidence was higher; peripheral neuropathy occurred in 66%, fatigue in 52%, nausea in 50%, diarrhea in 32%, arthralgia in 27%, and pyrexia in 25%. The occurrence of peripheral neuropathy appeared to be more significant in this study, with 14% of patients reporting grade 3 peripheral neuropathy and 8 patients discontinuing treatment owing to this adverse event. The objective response rate was also slightly higher among both the overall treatment population (59%, with 34% complete remissions) and patients treated at the maximum tolerated dose (58%).

The weekly dosage schedule did not appear to significantly increase the overall activity of brentuximab vedotin, but it was associated with a high incidence of peripheral neuropathy. Therefore, a schedule of once every 3 weeks was chosen for continued development in 2 phase 2 trials.<sup>12,13</sup> Both trials were single-arm, multicenter studies that administered up to 16 cycles of single-agent brentuximab vedotin at a dose of 1.8 mg/kg once every 3 weeks. Patients with relapsed or refractory CD30-expressing lymphoma (either Hodgkin lymphoma or systemic ALCL) were enrolled, and the primary endpoint was the overall objective response rate, as determined by an independent central review facility.

In the first phase 2 study, which enrolled 102 patients with Hodgkin lymphoma, the vast majority (94%) exhibited tumor reductions, and the disease control rate (complete remission, partial remission, or stable disease) was 96%.<sup>12</sup> The median progression-free survival was 7.8 months (Figure 5). An objective response rate of 75% was reported, of



**Figure 5.** In a phase 2 study of 102 patients with Hodgkin lymphoma, brentuximab vedotin was associated with a progression-free survival of 7.8 months. Adapted from Younes A et al. *J Clin Oncol.* 2012;30:2183-2189.<sup>12</sup>

which 34% were complete remissions. Overall, the median duration of response was 6.7 months, but it was markedly prolonged (to 20.5 months) among patients with a complete remission. More than half (55%) of patients experienced significant toxicity (an adverse event of grade 3 or higher), including nonfebrile neutropenia (20%), peripheral sensory neuropathy (8%), thrombocytopenia (8%), and anemia (6%). Adverse events led to dose delays in 47% of patients. The most frequently reported treatment-related adverse events of all grades included peripheral sensory neuropathy (42%), nausea (35%), fatigue (34%), neutropenia (19%), diarrhea (18%), pyrexia (14%), vomiting (13%), arthralgia (12%), pruritus (12%), myalgia (11%), peripheral motor neuropathy (11%), and alopecia (10%).

The second phase 2 study enrolled 58 patients with systemic ALCL (28% and 72% with ALK-positive and ALK-negative disease, respectively).<sup>13</sup> Again, a high number of patients achieved tumor reductions (97%), and the objective response rate was 86% (including 57% complete remissions). ALK status of the disease did not seem to significantly impact treatment response, as a similar proportion of ALK-positive and ALK-negative patients achieved an objective response (81% and 88%, respectively), and the median duration of response did not differ significantly between the

2 groups. Grade 3 or higher adverse events were reported in 60% of patients, including nonfebrile neutropenia (21%), thrombocytopenia (14%), peripheral sensory neuropathy (12%), and anemia (7%). Approximately one-quarter (24%) of patients discontinued treatment because of adverse events, and 40% required a delay in dosing. The most frequently reported treatment-related adverse events of all grades included peripheral sensory neuropathy (41%), nausea (40%), fatigue (38%), pyrexia (34%), diarrhea (29%), rash (24%), constipation (22%), and neutropenia (21%).

Based on the positive activity and well tolerated toxicity profile in these multicenter phase 2 trials, the US Food and Drug Administration granted accelerated approval to brentuximab vedotin for 2 indications.<sup>14</sup> The first is for the treatment of patients with Hodgkin lymphoma after failure of autologous stem cell transplantation (ASCT) or after failure of at least 2 prior multiagent chemotherapy regimens in patients who are not transplant candidates. The second indication is for the treatment of patients with systemic ALCL after failure of at least 1 prior multiagent chemotherapy regimen. The original approval allowed continued treatment up to a maximum of 16 cycles; in 2013, the duration was extended to allow treatment until disease progression or unacceptable toxicity.



## Clinical Trials of Brentuximab Vedotin That May Impact Standard of Care in Hodgkin Lymphoma

Several clinical trials are investigating brentuximab vedotin alone or as part of a combination chemotherapy regimen. The addition of brentuximab vedotin to chemotherapy may improve the response rate of either treatment alone or increase the rate of complete remission or duration of response. Brentuximab vedotin may also provide a better tolerated alternative to a more toxic regimen.

### *Before Allo-SCT*

Chen and colleagues conducted a retrospective analysis of patients with relapsed or refractory Hodgkin lymphoma who were treated with brentuximab vedotin in clinical trials and then subsequently underwent reduced-intensity allogeneic hematopoietic stem cell transplantation (allo-SCT).<sup>15</sup> Among the 18 patients, 17 had undergone previous ASCT. This small study of heavily pretreated relapsed/refractory Hodgkin lymphoma patients concluded that brentuximab vedotin may be an effective and less toxic treatment allowing for this reduced-intensity procedure.

### *Relapsed/Refractory*

An ongoing phase 1 clinical trial is assessing the combination of brentuximab vedotin with temsirolimus in patients with relapsed or refractory Hodgkin lymphoma after failure of ASCT or at least 2 prior cytotoxic chemotherapy regimens.<sup>16</sup> This dose-escalation study will permit treatment for up to 16 cycles.

A phase 1/2 multicenter study is under way to assess the safety and effectiveness of brentuximab vedotin plus bendamustine in patients with Hodgkin lymphoma or ALCL that has either relapsed or did not respond to initial treatment.<sup>17</sup> Patients with Hodgkin lymphoma are eligible if they failed or declined ASCT; patients who are not candidates for ASCT must have failed at least 2 prior multiagent chemotherapy regimens. Patients with ALCL are eligible if they failed at least 1 prior multiagent chemotherapy regimen and if they are not eligible for ASCT or declined it.

One of the most widely used chemotherapy regimens in the salvage therapy setting is ifosfamide, carboplatin, and etoposide (ICE). ICE chemotherapy is associated with numerous side effects, and typically requires the patient to be hospitalized for treatment. Response to ICE is often monitored by positron emission tomography (PET) scans, which can be used to measure abnormal metabolic activity in the disease. The goal of treatment is to achieve a negative PET scan (no abnormal activity) prior to ASCT, as this result predicts a better outcome after transplant. One clinical trial is evaluating the use of brentuximab vedotin instead of ICE in this setting, as a way to achieve complete remission with less toxicity prior to stem cell

transplantation.<sup>18</sup> Patients with no abnormal activity on PET scan will go on to receive stem cell transplantation without receiving ICE. Cases with evidence of abnormal activity by PET scan will undergo further treatment options, such as ICE chemotherapy. Although this study is ongoing, early reported results provided evidence that several patients were able to achieve a complete remission with brentuximab vedotin salvage therapy alone.<sup>19</sup>

### *ASCT Consolidation*

AETHERA (ADC Empowered Trial for Hodgkin to Evaluate PRogression After Autologous SCT) is an ongoing randomized, double-blind, placebo-controlled, phase 3 clinical trial evaluating the efficacy of brentuximab vedotin in patients at high risk of residual Hodgkin lymphoma following ASCT.<sup>20</sup> In this study, patients who underwent transplantation were randomized to treatment with either brentuximab vedotin or placebo, both given with best supportive care. The primary study endpoint is progression-free survival, and overall survival is among the secondary endpoints.

### *Frontline*

ECHELON-1 (Phase 3 Frontline Therapy Trial in Patients With Advanced Classical Hodgkin Lymphoma) is an ongoing open-label, multicenter, phase 3 trial. It is randomizing patients with Hodgkin lymphoma to frontline treatment with either standard chemotherapy (doxorubicin, bleomycin, vinblastine, and dacarbazine) alone or a similar chemotherapy regimen (doxorubicin, vinblastine, and dacarbazine) plus brentuximab vedotin.<sup>21</sup> The primary objective of this study is modified progression-free survival; overall survival is a secondary endpoint. The design of this study incorporated results from a phase 1 trial that found excessive formulary toxicity with the combination of bleomycin and brentuximab vedotin<sup>22</sup>; this finding led to the elimination of bleomycin from the combination regimen for the phase 3 trial.

## Evaluation of Brentuximab Vedotin in Other CD30-Expressing Lymphoid Malignancies

Subsets of other lymphoid malignancies have also been shown to express CD30, including primary mediastinal large B-cell lymphomas (a subset of diffuse large B-cell lymphoma), peripheral T-cell lymphoma not otherwise specified, and certain cutaneous malignancies, such as cutaneous ALCL, mycosis fungoides, and lymphomatoid papulosis. CD30 expression in these other malignancies is lower than it is in Hodgkin lymphoma and ALCL, which seems to correlate with a generally lower response rate to brentuximab vedotin in these cancers. CD30 may eventually be viewed as a biomarker for management in these malignancies, whereby the expression of this antigen may be used to select treatments such as brentuximab vedotin.

### **Frontline Setting in Mature CD30-Expressing T-Cell Malignancies**

ECHELON-2 (A Comparison of Brentuximab Vedotin and CHP With Standard-of-Care CHOP in the Treatment of Patients With CD30-Positive Mature T-Cell Lymphomas) is a double-blind, randomized, multicenter study designed to compare the efficacy and safety of brentuximab vedotin in combination with cyclophosphamide, doxorubicin, and prednisone vs cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) alone, both given in the frontline setting.<sup>23</sup> The regimen of cyclophosphamide, doxorubicin, and prednisone is used with brentuximab vedotin because of the potential for overlapping neurotoxicity between this agent and vincristine. This study will include CD30-positive patients with mature T-cell lymphomas, such as ALCL, non-Hodgkin lymphoma, and other T-cell lymphomas. Patients are designated as CD30-positive if at least 10% of neoplastic cells are expressing CD30. Progression-free survival is the primary study endpoint, and objective response rate and overall survival are among the secondary endpoints.

### **CD30-Expressing Relapsed or Refractory Lymphoid Malignancies**

An open-label, multicenter, phase 2 clinical trial is currently under way to investigate the safety and efficacy of brentuximab vedotin in patients with other CD30-expressing relapsed or refractory lymphoid malignancies.<sup>24</sup> This study consists of 2 parts—the first is designed to assess single-agent brentuximab vedotin in CD30-expressing non-Hodgkin lymphoma, and the second will evaluate brentuximab vedotin in combination with rituximab for treatment of diffuse large B-cell lymphoma. The combination of brentuximab vedotin with rituximab plus CHOP is also being tested as a frontline therapy in a randomized phase 2 trial of patients with diffuse large B-cell lymphoma.<sup>25</sup> Brentuximab vedotin is also under evaluation for the treatment of CD30-expressing cutaneous T-cell lymphomas in an open-label phase 2 study.<sup>26</sup>

Nonlymphomatous malignancies have also been shown to express CD30. An ongoing open-label, multicenter, phase 2 trial is evaluating brentuximab vedotin as a single agent in patients with a variety of CD30-expressing nonlymphomatous malignancies.<sup>27</sup> Patients will receive 1 of 3 different dose levels of brentuximab vedotin in this study—1.8 mg/kg once every 3 weeks; 2.4 mg/kg once every 3 weeks, or 1.2 mg/kg weekly for 3 weeks of a 4-week cycle. An early assessment of 3 patients with relapsed or refractory CD30-expressing testicular cancer who were treated on this study was recently reported, suggesting that brentuximab vedotin was safe and active in these patients.<sup>28</sup> Advanced and platinum-resistant CD30-expressing germline tumors are the specific target in an open-label phase 2 trial in which patients will receive the agent as a salvage therapy.<sup>29</sup>

### **Relapsed Cutaneous T-Cell Lymphoma**

The ALCANZA (Phase 3 Study of Brentuximab Vedotin Versus Physician's Choice of Methotrexate or Bexarotene in Patients [pts] With CD30-Positive [CD30+] Cutaneous T-Cell Lymphoma) trial is a phase 3 clinical trial of brentuximab vedotin for patients with relapsed cutaneous T-cell lymphoma in whom at least 10% of neoplastic cells express CD30.<sup>30</sup> The primary endpoint is overall response rate lasting at least 4 months. It is expected that approximately 124 patients will be enrolled.

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## General Discussion

**Eduardo M. Sotomayor, MD** In discussions about CD30, a question always arises regarding how immunohistochemistry compares to flow cytometry. There are very good antibodies for both. What are your thoughts regarding the differences?

**Ken H. Young, MD, PhD** We have compared both of these methods in acute myelogenous leukemia and acute lymphoblastic leukemia patients, and overall they produce highly consistent results. However, we have very few samples from lymphoma patients available for flow cytometry analysis because immunostaining is part of the regular workup instead.

**Eduardo M. Sotomayor, MD** What about cases of patients with follicular lymphoma or ALCL who have bone marrow involvement?

**Ken H. Young, MD, PhD** In that situation, we could run both flow cytometry and immunostaining.

**Eduardo M. Sotomayor, MD** Do you favor one over the other in patients with B-cell lymphomas?

**Ken H. Young, MD, PhD** In the academic setting, where there is ready access to an expert hematopathologist, flow cytometry is much more rapid, with typical turnaround times of approximately 5 hours. In contrast, immunostaining can take up to 1 to 2 days. The relative times for both are longer in the community setting, as these labs routinely send these samples out to a reference laboratory (in the case of flow cytometry) or to a nearby

academic center (in the case of immunostaining), but the answer remains the same—flow cytometry has a faster turnaround time. In general, flow cytometry would be preferred not only because it provides a more rapid answer but also because it is much more quantitative to provide a percentage of positive cells in a particular group of cells.

**Eduardo M. Sotomayor, MD** As we have already discussed, activation of CD30 (like other TNFRs) can exert pleiotropic effects in cells. Given this activity, I found the data regarding diffuse large B-cell lymphoma patients with Epstein-Barr virus to be very interesting.<sup>1</sup> It seems that patients with diffuse large B-cell lymphoma that is positive for both CD30 and the Epstein-Barr virus have a poor outcome.

**Ken H. Young, MD, PhD** Yes, this specific group of diffuse large B-cell lymphoma patients has a poorer survival. The outcome becomes much better in patients with CD30 expression who are negative for the Epstein-Barr virus infection.

**Eduardo M. Sotomayor, MD** Have you investigated the different CD30-related signaling pathways in these 2 settings?

**Ken H. Young, MD, PhD** I have not, but such a study would be very valuable. We have evaluated the gene expression profile and analyzed the differences between these 2 groups for several critical lymphoma-associated biomarkers and translocations.<sup>2</sup> These investigations did provide evidence that 2 signaling pathways, the NF- $\kappa$ B and the JAK-STAT, are significantly different in these 2 settings. In a separate



study, we found that STAT3 expression correlated with poor survival.<sup>3</sup> However, we have not evaluated whether STAT3 is a specific signaling pathway that is linked to or regulated by CD30 expression. A recent whole exome sequencing study completed by my team shows genomic difference between these 2 groups of lymphoma.

**Eduardo M. Sotomayor, MD** In Epstein-Barr virus-positive disease, it might be predicted that the viral infection is driving CD30 expression. In this subset, NF- $\kappa$ B becomes overactivated, conferring a survival advantage to the cells. I wonder if the apoptotic pathways are suppressed in these cells.

**Ken H. Young, MD** We have not yet looked at this notion. There may or may not be independent activation of apoptosis genes, such as those in the Bcl-2 family.

**Eduardo M. Sotomayor, MD** Have you evaluated CD30 expression and outcomes in virus-driven T-cell lymphomas?

**Ken H. Young, MD, PhD** T-cell lymphoma outcomes are still debatable. For example, a French study reported slightly better survival among CD30-positive T-cell lymphomas vs their CD30-negative counterparts.<sup>4</sup> Other studies demonstrate that CD30 expression is a poor prognostic factor in patients with malignancies including adult T-cell leukemia/lymphoma, NK lymphoma, and enteropathy-associated T-cell lymphoma.<sup>5</sup> The role and prognostic impact of CD30 expression in different subtypes of T-cell lymphoma should be evaluated independently owing to differences in cell of origin, biology, phenotype, genomics, and treatment response.

**Eduardo M. Sotomayor, MD** So it seems that, overall, CD30-positive, virally driven lymphomas have a poor outcome.

**Ken H. Young, MD, PhD** Correct. In addition, there is also evidence that CD30 expression is a poor prognostic indicator in some patients with transformed or recurrent high-grade malignant lymphoma. However, no reliable study has been performed. My team will complete such studies in recurrent and transformed high-grade lymphoma patients.

**Eduardo M. Sotomayor, MD** I wonder what is inducing the expression of CD30 in patients with transformed disease.

**Ken H. Young, MD** That is an important and interesting question that remains unanswered. For example, transformed diffuse large B-cell lymphoma may arise from follicular lymphoma or other low-grade B-cell lymphomas. Inactivation of tumor suppressor genes and activation of oncogenes, owing to genomic defects and/or epigenetic dysregulation, may result in abnormal CD30 signaling pathway transduction.

**Eduardo M. Sotomayor, MD** Have you ever looked at correlations with CD30 expression in large granular lymphocytic leukemia?

**Ken H. Young, MD** Yes, our preliminary study on several T-cell large granular lymphocytic leukemia patients showed that no CD30 expression was seen in this type of low-grade, indolent T-cell lymphoma/leukemia. It will be important to confirm this finding in a relatively large series of patients.

**Eduardo M. Sotomayor, MD** At the American Society of Hematology meeting in 2013, data were presented in which brentuximab vedotin was used in the treatment of patients with relapsed or refractory CD30-positive non-Hodgkin lymphoma.<sup>6</sup> In this group, approximately 50 patients had diffuse large B-cell lymphoma. CD30 expression was quite variable among these patients, with only 10% showing high expression and many patients showing only 10%, 20%, or 30% positivity. The overall response rate was 42% among the patients with diffuse large B-cell lymphoma, and the investigators concluded that responses could be observed across a range of CD30 expression types. For example, there was evidence of some patients with undetectable CD30 levels who nevertheless achieved a response to treatment. This finding raises many questions.

**Ken H. Young, MD, PhD** Yes, there is a realization that brentuximab vedotin can also be effective in some patients with CD30-negative lymphoid malignancy. The mechanism for this activity is unknown and worthy of further investigation.

**Eduardo M. Sotomayor, MD** What are the potential reasons why a supposedly CD30-negative patient would respond to treatment with brentuximab vedotin?

**Ken H. Young, MD, PhD** One possibility is that it is a false negative, and that the patient may instead have a high level of soluble CD30 in the blood. Another explanation is that the patient is truly CD30-negative, but can still respond to brentuximab vedotin through a different signaling pathway or biologic mechanism. The interaction of brentuximab vedotin with suboptimal CD30 antigens on the lymphoma cell surface may be sufficient to show therapeutic effects. It is also possible that brentuximab vedotin may exhibit its regulatory function and therapeutic effect on components of the tumor microenvironment, including macrophages, mesenchymal cells, cytokines and chemokines, and the stromal matrix.

**Eduardo M. Sotomayor, MD** One mechanism could be that the antibody-drug conjugate is binding the soluble CD30, resulting in the formation of a specific complex that might promote some sort of response. Brentuximab

vedotin is highly specific, and thus far there is no evidence of off-target effects. I think this finding raises the question of how to define CD30-negative status. Are these patients truly CD30-negative, or is there an error or artifact inherent to current CD30 detection methods?

**Ken H. Young, MD, PhD** Yes, it may be that there is some limitation that causes the biopsy to not be truly representative of the patient's disease, at least in terms of CD30 expression. We typically define staining of less than 20% as CD30-negative in diffuse large B-cell lymphoma. In other types of lymphoma, such as T-cell lymphoma and NK-cell lymphoma, the cutoff should be established independently in any specific study.

There are some cases in which there may be CD30 expression in few lymphoma cells, but the percentage is relatively low. In any type of lymphoma projects, the cutoff for CD30 expression should be established independently in correlation to treatment response and survival. Analyses using receiver operating curves and X-tile plots are commonly used and accepted in the research field. In addition, when a particular CD30 antibody is used for immunohistochemical staining, it is unknown if there is any mutation present in the antibody-binding site that will abolish the CD30 antibody interaction. Such phenomena have been seen in BCL2 immunostains, where the mutations are found in the BCL2 antibody-binding epitope site, causing a low frequency of positive staining. Mutation analysis on the CD30 gene may need to be studied in relevant types of cancer to address this question.

**Eduardo M. Sotomayor, MD** What about a case of diffuse large B-cell lymphoma that shows less than 20% CD30 staining in the lymph node? By your definition, this expression would be considered negative. But what if this patient also had bone marrow involvement that showed high levels of CD30 expression in the malignant B cells?

**Ken H. Young, MD, PhD** We would then consider the patient to be CD30-positive. In pathology and diagnostic practice, when a particular antigen expression differs in various cancer lesion sites, the higher expression is commonly used as the representative value. However, we have not seen any of those cases in our series of studies; most diffuse large B-cell lymphoma patients we have included for the study had bone marrow staging flow cytometry analysis that was negative for involvement. Your point about the discrepancy between samples in the same patient is important, and this phenomenon has not yet been addressed. From cancer biology studies, we have learned that the tumor microenvironment, which includes macrophages, mesenchymal cells, the stromal matrix, cytokines, and chemokines, may contribute to biomarker expression, drug resistance, and cancer progression.

I am also curious as to what the outcome is for patients with CD30 staining that is less than 20%. For example, does the outcome change for diffuse large B-cell lymphoma patients with 1% or 3% staining, as opposed to 18% or 19% staining? We have not quantitatively analyzed and compared these groups to each other, but it may be worth examining in more detail.

**Eduardo M. Sotomayor, MD** Is there any correlation between CD30 expression in the lymph nodes and PET activity?

**Ken H. Young, MD, PhD** We have not evaluated this important question in primary lymphoma patients, nor have we examined whether CD30 expression correlates with radiographic findings, clinical chemistry abnormalities, recurrence, or progression status. When PET and radiographic approaches are routinely used for lymphoma staging and diagnosis, this study will be important to perform. However, we may analyze primary de novo lymphoma patients separately from those transformed and recurrent patients. In the recurrent lymphoma patients who have undergone several treatment regimens in the past, biomarker and radiographic evaluations may be challenging and must be interpreted carefully.

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# Slide Library

## CD30

- A transmembrane glycoprotein receptor consisting of an extracellular domain, a transmembrane domain, and an intracellular domain
- The extracellular domain of CD30 contains 6 cysteine-rich stretches of amino acids, which help to form a scaffold of disulfide bonds, creating an elongated structure<sup>1</sup>
- The intracellular cytoplasmic tail domain contains several serine/threonine residues that can become phosphorylated, creating docking sites for adaptor proteins to bind to and transduce signaling into the cell

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## CD30 Expression in Malignant Cells

- Hodgkin lymphoma and ALCL are the 2 forms of lymphoma in which CD30 expression is present in nearly all patients<sup>1-4</sup>
- CD30 expression is reported in approximately 30% of T-cell malignancies<sup>5</sup>
- Between 15% and 20% of B-cell malignancies are reported to be CD30-positive<sup>6</sup>

1. Stein H et al. *Blood*. 1982;60(1):155-158. 2. Schwab U et al. *Blood*. 1982;79(7670):45-47.  
3. Stein H et al. *Int J Cancer*. 1982;30(3):361-365. 4. Stein H et al. *Blood*. 2000;95(1):144-148.  
5. Lee S-S et al. *Int J Cancer*. 2003;105(1):13-20. 6. Lee J et al. *Blood*. 2005;105(1):144-148.

## Tools for Detection of CD30 Expression

- Immunohistochemical staining (IHC)
- Enzyme-linked immunosorbent assay (ELISA)
- Flow cytometry

## Interpretation of IHC Staining

Interpretation of IHC staining is significantly impacted by:

- The tissue fixation process, including the fixation duration
- Type of fixatives
- Antigen retrieval
- Antibody staining procedure
- Quality of tissue
- The presence of any crush artifacts or necrotic changes

## Targeting CD30

- The antibody-drug conjugate brentuximab vedotin consists of the naked anti-CD30 antibody 5G8-30 conjugated to the synthetic antitubulin agent MMAE
- The 2 moieties in brentuximab vedotin are conjugated via a dipeptide linker that is cleaved by lysosomal enzymes upon receptor-mediated internalization into target cells<sup>1-4</sup>
- Once released, MMAE induces cell growth arrest followed by apoptosis<sup>1,4</sup>

1. Chao R et al. *Cancer Therapy*. 2011;17(15):16. 2. Swendsen R et al. *Clin Cancer Res*. 2005;11(2):411-421. 3. Chizzola E et al. *Int J Hematol*. 2009;102(1):9-12. 4. Francisco J et al. *Blood*. 2003;101(1):145-149.

## Brentuximab Vedotin: Clinical Data

- In a phase 2 study of 102 patients with Hodgkin lymphoma, 94% exhibited tumor reductions, and the disease control rate was 96%.<sup>1</sup> The median progression-free survival was 7.8 months.
- In a phase 2 study of 58 patients with systemic ALCL, 97% exhibited tumor reductions, and the objective response rate was 86%.<sup>2</sup>

1. Younes A et al. *J Clin Oncol*. 2012;30(18):2183-2189. 2. Phu B et al. *J Clin Oncol*. 2012;30(18):2190-2196.

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