Q&A column

Q. Is CD30 currently being used as a predictive marker for therapy?

A. CD30 is a transmembrane phosphorylated glycoprotein and a member of the tumor necrosis factor receptor superfamily 8 (TNFRSF8). In the hematopoietic system, CD30 is expressed on normal activated T and B cells, as well as virally transformed T and B cells. Monocytes/macrophages and granulocytes also constitutively express CD30. In lymph node and tonsil sections a small subset of lymphocytes in the parafollicular areas express CD30. CD30 is thought to transduce a cell survival signal and be involved in the T cell dependent portion of the immune response. CD30 staining may be membranous or concentrated in the Golgi zone outside the nucleus (paranuclear).

CD30 is consistently overexpressed on Hodgkin/Reed-Sternberg cells of classic Hodgkin lymphoma (CHL). However, CD30 expression is seen in a number of other neoplasms including: CD30 positive cutaneous lymphoproliferative disorders, systemic anaplastic large cell lymphoma (ALCL), adult T cell lymphomas, a subset of peripheral T cell lymphomas, most cases of transformed mycosis fungoides, a subset of B cell lymphomas (mostly diffuse large B cell lymphoma; DLBCL), and a subset of germ cell tumors (GCT) including embryonal carcinoma. CD30 expression in neoplasms has become of increasing importance with the development of and successful treatment with anti-CD30 monoclonal antibodies. Original attempts at anti-CD30 were unsuccessful as naked CD30 antibodies were rapidly endocytosed. Brentuximab vedotin is an anti-CD30 chimeric monoclonal antibody that is conjugated to the antimicrotubule agent monomethyl auristatin E. Brentuximab vedotin may be used to target a variety of CD30-expressing neoplasms (see box below). It is important to keep in mind that an unsatisfactory CD30 assessment by immunohistochemistry can be a barrier in identifying appropriate patients for CD30-targeted therapy.[1] Interestingly, NordiQC had four runs for CD30 assessment over a 10-year period.[2] CD30 staining assessment by NordiQC has shown a steady decline in achieving sufficient staining results, decreasing from 92 percent to 71 percent in 2015.

CD30-expressing neoplasms

- Classic Hodgkin lymphoma
- Systemic and primary cutaneous anaplastic large cell lymphoma
- CD30 positive cutaneous lymphoproliferative disorders
- Diffuse large B cell lymphoma
- Peripheral T cell lymphoma, NOS
- Transformed mycosis fungoides
- Germ cell tumors

The use of CD30 as a predictive marker for therapy is relatively young. While in most of the tumors treated with brentuximab vedotin the CD30 expression is constitutive (for example, CHL, ALCL, GCT), there has been no well-established cutoff for percent or intensity of CD30 expression in tumor cells that leads to an effective response. In one article on expression of CD30 in DLBCL, a cutoff of ≥five percent was suggested.[3]

1. Wasik MA, Jimenez GS, Weisenburger DD. Targeting CD30 in malignant tissues: challenges in detection and clinical applications. *Pathobiology*. 2013;80(5):252–258.
2. Nordic Immunohistochemistry Quality Control, CD30 assessment runs 11 (2004;11_5.pdf), 25 (2009;25_5.pdf), 31 (2011;31_5.pdf), 43 (2015;43_5.pdf). NordiQC website. www.nordiqc.org/downloads/ assessments. Accessed May 16, 2018.
3. Naeini YB, Wu A, O'Malley DP. Aggressive B-cell lymphomas: frequency, immunophenotype, and genetics in a reference laboratory population. *Ann Diagn Pathol*.

2016;25:7–14.

Dennis P. O'Malley, MD
Pathologist, NeoGenomics
Aliso Viejo, Calif.
Member, CAP Immunohistochemistry Committee

Mohamed El-Sayed Salama, MD
Medical Director, Mayo Medical Laboratories, Mayo Clinic, Rochester, Minn.
Member, CAP Immunohistochemistry Committee

Q. Due to laboratory construction, our molecular instruments were relocated within the lab. Is full test validation required in this case? Or is running at least 20 known samples enough to verify the instrument/assay performance specifications?

A. It is the laboratory's responsibility to ensure that instruments function properly and that performance is not affected when the instruments are moved to another location. Not all moves are the same, and different types of moves may require more extensive checks and reverification or revalidation processes. The relocation process itself could cause damage, even if short distances are involved. The new location could subject instruments to different environmental conditions (e.g. temperature, humidity, ventilation, sunlight) or other factors (e.g. new water source, different types of personnel, cross-contamination) that could affect performance. For molecular testing using nucleic acid amplification, the laboratory must also consider the potential for amplicon contamination and the need for adequate physical separation of pre- and post-amplification processes. Some moves may involve an extended downtime that could have a negative impact on an instrument. When relocating an instrument, laboratories should refer to the manufacturer's manual for critical requirements for setup, limitations, and environmental conditions. The laboratory may also wish to contact the manufacturer for further recommendations.

Before performing a reverification or revalidation study to confirm that the method performance specifications were not affected, the laboratory first needs to ensure that the move has not had an impact on operational performance. Typical steps would include completion of maintenance and instrument function checks following the manufacturer's instructions, including startup and calibration processes. After the laboratory determines that the instrument is operating properly, the laboratory must reverify or revalidate the method performance specifications (e.g. accuracy, precision, reportable range) in the location in which testing will be performed. Confirming that the move has not affected performance may not require a process as extensive as the initial method verification or validation process. The number of samples to be used is to be determined by the laboratory based on the extent of the move and other factors that may have changed. Records of the reverification or revalidation must be available upon request during an inspection.

1. Clinical and Laboratory Standards Institute. MM19-A: Establishing Molecular Testing in Clinical Laboratory Environments; Approved Guideline, 2016.
2. U.S. Department of Health and Human Services, Centers for Medicare and Medicaid Services. Clinical Laboratory Improvement Amendments of 1988; final rule. *Fed Regist.* 1992; 7146 42 CFR §493.1253.
3. College of American Pathologists. COM.30550 Instrument/equipment performance verification; COM.40000 Method validation

approval—nonwaived tests. In: All common checklist. Aug. 21, 2017.
4. College of American Pathologists. MOL. 35350 Carryover; MOL.30785 Validation summary; MOL.36015 NGS analytical wet bench process validation. In: Molecular pathology checklist. Aug. 21, 2017.
5. College of American Pathologists. MIC. 65500 Carryover. In: Microbiology checklist. Aug. 21, 2017.

Lyn Wielgos, MT(ASCP), Checklist Editor, CAP Accreditation Programs
College of American Pathologists, Northfield, Ill.