Society for Hematopathology

Webinar Q&A: April 29, 2026

"ALK-negative Anaplastic Large Cell Lymphoma: Diagnostic Challenges, Differential Diagnosis, and Molecular Pathogenesis"

Speaker:
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1. **Question**: ALK- ALCL appears quite heterogeneous. How to differentiate it from PTCL NOS with prominent CD30 expression? Esp. with DUSP22-R?
   Answer: When PTCL NOS shows prominent CD30 expression, distinguishing between ALK-negative ALCL and CD30+ PTCL NOS can be challenging. The presence of hallmark cells, a cohesive or sinusoidal growth pattern, strong and uniform CD30 expression with membranous and Golgi pattern and positivity for cytotoxic markers, EMA and clusterin support a diagnosis of ALK-negative ALCL. However, *DUSP22*-rearranged ALK-negative ALCL cases show unique morphological (smaller cell size, less hallmark cells) and immunophenotypic features (no or minimal expression of cytotoxic markers and EMA), and may be misdiagnosed as PTCL NOS. But hallmark cells are still present. In addition, the monotonous cells and donut cells in some areas and CD15 expression are hints for *DUSP22*-rearranged ALCL cases. Lack of loss of T-cell antigens favor PTCL, NOS.

2. **Question:** What are the diagnostic clues to distinguish CD30 positive PTCL from ALK negative ALCL and does it differ in management?
   Answer: please see the answer to Question #1. The treatment for ALK-negative ALCL and CD30+ PTCL NOS is different. Also, ALK-negative ALCL patients often have better clinical outcomes.

3. **Question:** For flow cytometry for ALCL, what are your suggestions in handling the compensation and autofluorescence for immunophenotyping?
   Answer: The channel for CD30 (we use PE) needs to do specific comp, often using cells with bright expression of CD30 when setting up the panel. ALCL is usually very bright for CD30 and never confused with autofluorescence background. Autofluorescence becomes relevant in cases with partial or dim CD30 expression (such as post Brentuximab treatment). To assess background, use: (1) internal negative populations with similar FSC/SSC (for example monocytes or granulocytes in the same tube), and (2)

cells from other tubes where the PE channel marker is negative to establish baseline signal.

4. **Question:** Would you diagnose ALK- ALCL without any T-cell marker expression, negative CD45 - only based on T-cell clonality being positive and presence of JAK2 rearrangement?
   Answer: No. ALK-negative ALCL is rarely negative for CD45. T cell clonality by PCR may not be reliable. *JAK2* rearrangement is not specific either and can be detected in myeloid and lymphoid neoplasm (such as T cell lymphoma, CHL). Morphology and immunophenotype are very important in making diagnosis.

5. **Question:** Is there a difference between "uniform, strong" and "diffuse, strong" CD30 expression (diff ALK- ALCL vs CD30+ NK/TCL)?
   Answer: There may be some minor variations in density when describing “diffuse, strong” expression.

6. **Question:** Can PTCL, NOS show EMA and mum1 expression?
   Answer: PTCL NOS is usually negative for EMA. A small subset of PTCL NOS cases is positive for MUM1.

7. **Question:** It looks like a reliable morphologic feature of ALCL is cohesive growth. If possible - could you show examples of ALCL and not-ALCL, and describe the difference in growth patterns?
   Answer: ALCL is characterized by cohesive growth pattern (like carcinoma).

8. **Question:** How do you differentiate CD30+ PTCL vs. ALK- ALCL?
   Answer: please see the answer to Question #1.

9. **Question:** Did you test for HTLV1 in case 6 (leukemic ALCL)?
   Answer: Yes, it is negative.

10. **Question:** In the setting of bone marrow evaluation after initial diagnosis of ALK-ALCL, would you mind suggest the striking feature of bone marrow involvement by the lesion and how to differentiate them using CD30 (in case of some subtle involvement).

Answer: For the staging marrow of ALK-negative ALCL cases, if it is subtle or negative by morphology, we usually do CD30 IHC.

11. **Question:** For case 7, myeloid sarcoma, what's the explanation for the positive T-cell clonality?
    Answer: T-cell clonality can be seen in myeloid neoplasms (such as MDS, AML), which may be due to the clonal expansion of T cells driven by immune responses to tumor antigens.

12. **Question:** Have you encountered or diagnosed cases of ALCL with weak or non-diffuse CD30 expression?
    Answer: If the patient did not receive Brentuximab (anti-CD30), uniform and strong CD30 expression is required for a diagnosis of ALK-negative ALCL. If the patient has been treated with Brentuximab, CD30 expression on ALK-negative ALCL cells may become weak, subset, and even negative.

13. **Question:** Can ALK- ALCL be EBER+?
    Answer: Per the most recent WHO/ICC, ALK-negative ALCL is negative for EBER.

14. **Question:** Have you seen cases of ALK negative ALCL, with EBER positive among the smaller background cells and negative among the large, atypical cells?
    Answer: I did not see such cases. If you see EBER+ only in the smaller background cells (not lymphoma cells), it might be a passenger virus in background reactive cells.

15. **Question:** For the GCT, did I hear correctly that the case is positive for TCR gene rearrangement study? If so, how would you explain this result? Pseudo-clone or?
    Answer: Yes, it is positive for *TRG* gene rearrangement. In combination with the expression of several T markers, we hypothesize this relapsed GCT may be undergoing malignant somatic (non-germ cell) transformation to a hybrid of GCT and T cell lymphoma.

16. **Question:** How often have you seen flow cytometry fail to detect clonal cells in ACLC cases? And if the flow comes back negative for neoplastic cells, what may be the reasons (aside from gating error that was mentioned in the lecture)?

Answer: Aside from gating errors, flow cytometry may occasionally fail to detect ALCL. This is uncommon but typically relates to technical or sampling issues, including: poor sampling (non-representative specimen), and very low level involvement (for example, scattered neoplastic cells in bone marrow). Rarely, tumor cells may be fragile or underrepresented in suspension, further reducing detection. (please see more details in Xu J, et al. Chapter 19, Flow Cytometry in Hematolymphoid Neoplasms edited by Wei Wang and Sa A Wang 2026)

17. **Question:** Since EBV negativity is listed as one of the WHO essential diagnostic criteria for ALK-negative ALCL, do we need to perform an EBV stain in every case in which ALCL is suspected, or only in CD56-positive cases is enough?
    Answer: I recommend performing EBER in every case.

18. **Question:** What may be your opinion on DUSP22-R and ALK-ALCL prognosis?
    Answer: The prognostic significance of *DUSP22-R* in ALK-negative ALCL is still controversial. Although *DUSP22*-R has been associated with a favorable outcome in systemic ALK-negative ALCL by some studies, our study of 81 ALK-negative ALCL patients and other studies showed that *DUSP22-R* was not associated with a better clinical outcome (PMID: 36453104).

19. **Question:** Can ALK negative ALCL be negative for T-cell gene rearrangement (T-cell clonality studies). Do you have any experience with PAX5+ expression in a subset of ALK-ALCL?
    Answer: I believe ALCL can be negative for TCR gene rearrangement. We occasionally see PAX5 expression (weak, subset) in ALK-negative ALCL. This aberrant expression of PAX5 may be associated with extra copies of the *PAX5* gene (PMID: 20118907).

20. **Question:** Have you encountered or diagnosed cases of ALCL with weak or non-diffuse CD30 expression?
    Answer: please see the answer to question #12.

21. **Question:** Is there CD30+ PTCL NOS with DUSP22-R?
    Answer: In a study of CD30+CD15+ PTCL NOS, 3 cases previously classified as PTCL showed *DUSP22* rearrangements, favoring a diagnosis of ALK-negative ALCL. The authors

suggest that cases previously designated CD30+CD15+ PTCL, likely fall within the spectrum of ALK-negative ALCL (PMID: 38613165).

22. **Question:** Do the features differentiating ALK-neg ALCL from PTCL, NOS hold true for distinguishing primary cutaneous ALCL from primary cutaneous PTCL, NOS?
    Answer: I do not have much experience in cutaneous cases.

23. **Question:** What's the % for diffuse uniform expression of CD30? >90%?
    Answer: Yes.

24. **Question:** Do you have any experience for LEF1 surrogate? Is it sensitive or specific?
    Answer: Every new ALK-negative ALCL case in our institution will be tested for *DUSP22*-R by FISH. I do not have any experience in LEF1 surrogate. The strong and uniform LEF1 expression pattern was reported to have a high positive predictive value (93.8%) and high negative predictive value (96%) for *DUSP22* rearrangement in ALK-negative ALCL (PMID: 33165091).

25. **Question:** I have a case of lung mass showing plenty of anaplastic large cell with dense neutrophilic and eosinophil background. IHC profile LCA, CD20 CD3,CD4,CD8,CD15,ALK1, PAX5, OCT2, BOB1 negative. Uniform CD30 expression and faint bcl6 positive. Can you please suggest how to proceed in this scenario, when I have a differential between Hodgkin and ALCL ALK - we have limited access to molecular /Fish Studies.
    Answer: It can be difficult to distinguish ALK-negative ALCL and CHL. ALCL can lose T-cell antigens and aberrantly express B-cell makers (CD19, CD79a and PAX5). CHL cases are usually negative for B-cell antigens except PAX5 (weak) and can aberrantly express T-cell markers and cytotoxic markers. Positivity for CD45, CD43, EMA and clusterin, and negativity for EBER favor ALK-negative ALCL.