Society for Hematopathology

Webinar Q&A: January 21, 2026

"Histiocytic Neoplasms: Morphologic Spectrum, Immunophenotypic Profiles, and Molecular Insights"

Speaker:
Dr. Aishwarya Ravindran
The University of Alabama at Birmingham

1. **Question**: **But I always see cyclin D1 positivity in even benign histocytes. How do we ddx between benign and malignant using cyclin D1?**

   Answer: Neoplastic histiocytes typically show uniform cyclinD1 expression (strong nuclear and weak to intermediate cytoplasmic staining) [reference PMID: 41495601; PMID: 37167084; PMID: 28622183]. In normal/reactive histiocytes, the nuclear pattern of cyclinD1 staining (when present) is variable with a majority being negative (refer to slide#41: green arrows point to reactive sinus histiocytes in the lymph node).

2. **Question: #13, for NGS, the lesional cells here tend to be very few, esp given the prevailing non-neoplastic inflammatory background. to complicate matter, macrodissection seems difficult, given they intermix. Any recommendations to do NGS?**

   Answer: This has always been a practical challenge, especially in RDD. Unfortunately, at this point in time, there is no commercially available testing kit for tumor enrichment in clinical practice.

   In our practice, in cases with negative NGS, we review the raw NGS data with our molecular geneticist to identify variants especially in the MAPK pathway with VAF <5%. Most commercial NGS report variants with VAF $\geq$5%, and identification of low VAF pathogenic variants is still clinically relevant for therapeutic purposes.

3. **Question: Do you have data with use of ALK1 or D5F3 clone for ALK+ histiocytosis?**

   Answer: True cases of ALK+ histiocytosis are exceedingly rare. In my 3 years of clinical practice at UAB, I have diagnosed only 2 cases of ALK+ histiocytosis, one of which was shown on slide #19 in the presentation. In these cases, two different ALK antibody clones (5A4 and D5F3) showed uniform cytoplasmic immunoreactivity without nuclear

expression, with subsequent NGS confirming *KIF5B::ALK* fusion. The largest published series on ALK+ histiocytosis details the use of various ALK IHC antibody clones including ALK01, ALK1, D5F3, 5A4 and 1A4 in supplemental table 1 (reference PMID: 34727172)

In my clinical practice, I have observed non-specific immunoreactivity with ALK-D5F3 antibody clone in reactive histiocytes associated with inflammatory skin disorders as well as macrophage-rich lesions in the brain. The pattern of staining is usually nuclear, and sometimes nuclear and cytoplasmic. However, on repeating the ALK IHC using a different antibody clone such as 5A4, such non-specific immunoreactivity was absent. These cases also lack cyclinD1 expression, unlike true ALK-positive histiocytosis which shows cyclinD1-positivity (reference PMID 34727172 - supplementary table 1).

4. **Question: Have you systematically evaluated ALK expression using D5 clone and ATI in established reactive histiocytic proliferations?**

Answer: I have only applied this in one case of reactive histiocytic proliferation which was shown during the presentation where we pursued RNA sequencing for ALK^{ATI} due to the presence of immunoreactivity by two different ALK antibody clones (D5F3 (nuclear and cytoplasmic) and 5A4 (predominantly cytoplasmic with weak nuclear expression); reference slides #21 and #23).

Pursuing RNA sequencing for ALK^{ATI} isoform expression is relevant in established cases of histiocytic neoplasms without ALK fusions but with ALK immunoreactivity, as detailed in the recent study (PMID: 41478363), as such cases may have therapeutic implications while considering potential ALK inhibitors.

5. **Question: How do you use cyclinD1? I find it's positive in all histiocytes, reactive or neoplastic.**

Answer: Pattern of cyclinD1 expression is the primary differentiating factor in neoplastic versus reactive histiocytes. CyclinD1 by itself is not a pathognomonic marker in histiocytic neoplasms but they should be used in conjunction with an IHC panel (S100, Factor 13a, CD1a, langerin, ALK, pan-TRK) to evaluate the histiocytic infiltrates. When there is a uniform pattern of cyclinD1 expression (nuclear and cytoplasmic), they are more suggestive of a neoplastic process rather than reactive histiocytic proliferations (reference PMID: 41495601; PMID: 41178439-table 1). Further, nuclear cyclinD1

expression is patchy/weak with a majority being negative in reactive histiocytes (refer to slide #41: green arrows point to reactive sinus histiocytes in the lymph node).

While typically different antibody clones for cyclinD1 IHC should not interfere with our assessment in histiocytic infiltrates, it would be helpful to analyze the pattern of cyclinD1 staining in your institutional cohort to determine the variant staining patterns in true neoplastic histiocytes versus reactive histiocytic proliferations.

6. **Question: How to call the histiocytic proliferation if the cytologic atypia is more than the usual appearance of RDD/LCH, mitosis+, but not pleomorphic enough for a straightforward malignant histiocytic neoplasm?**

   Answer: For cases with Langerhans cell phenotype, when the cytologic atypia is more than a typical LCH but insufficient to call MHN (i.e. Langerhans cell sarcoma), I prefer to use this term 'Langerhans cell neoplasm,' with a comment stating that these histopathologic features are insufficient for MHN.

   RDD cases can demonstrate occasional cytologic atypia, although the majority of the lesional histiocytes still show bland cytologic features. If it is more than the expected spectrum of cytologic atypia, then I would prefer not to subclassify this and label these as 'Histiocytic neoplasm, NOS/not further classifiable' (reference PMID: 41178439-Figure 4) with a comment stating why it does not fit into the category of RDD and is insufficient to classify as MHN.

7. **Question: For slide #53, which clone of PD-L1 are you using for these tumor?**

   Answer: The antibody clone for PD-L1 in the case represented on slide #53 was 22C3 (Dako). The antibody clone SP263 (Ventana Medical Systems) can also be used, when 22C3 is not available, based on our multi-institutional study on the utility of PD-L1 expression in MHNs (PMID: 40995654).

8. **Question: How do you differentiate between pleomorphic dermal sarcoma and histiocytic sarcoma on IHC basis?**

   Answer: PU.1 (slide #56) immunostaining is a helpful marker to distinguish true histiocytic lineage from other non-hematopoietic tumors including pleomorphic dermal

sarcomas (slides # 54-56). Pleomorphic dermal sarcomas are negative for lineage-defining markers including histiocytic markers (CD68, CD163, and PU.1).

CD68 (especially antibody clones other than PGM1) can be expressed in non-hematopoietic tumor cells; in such cases, a combination of histiocytic-lineage specific markers (CD163, CD4, PU.1) is often essential to exclude a histiocytic lineage. CD68 (cytoplasmic) and CD163 (cytoplasmic and membranous) mark admixed histiocytes which can show reactive atypia in non-hematopoietic tumors giving the false histologic impression of positivity in the tumor cells; in such scenarios, PU.1 (nuclear) can be helpful to delineate the non-hematopoietic tumor cells (PU.1-negative) from admixed reactive histocytes (PU.1-positive) [reference PMID: 37012662].

9. **Question: Is there a malignant counterpart for RDD? Like when the histiocytes look quite pleomorphic and emperipolesis present.**

   Answer: Yes, malignant histiocytic neoplasms, specifically the subcategory of interdigitating dendritic cell sarcoma (IDCS) can have RDD-like phenotype with diffuse strong S100 and OCT2 expression associated with emperipolesis. We have shown this example under the spectrum of 'monocyte-macrophage phenotype' in our prior study (PMID: 37406859- Figures 2 & 5; table 1).

10. **Question: For case #2, was LCH ruled out given the increased eosinophils. Is there a cut off of ALK positive staining needed in the tumor cells?**

    Answer: Immunostaining for CD1a was negative in case #2; a langerin IHC was not pursued given the CD1a-negativity.

    There is no defined cut-off for determining ALK-positivity in neoplastic histiocytes. Typically, the ALK immunoreactivity correlates with the extent of cyclinD1 expression in the neoplastic histiocytes (slides #19 and #20).

11. **Question: So can cyclin D1 reliably be used for IDCT versus reactive dendritic cell proliferations mimicking IDCT in the skin?**

    Answer: In my clinical practice, I have found cyclinD1 to be particularly helpful to distinguish reactive from neoplastic dendritic cell proliferation. Multiple studies have shown cyclinD1 to be particularly useful in differentiating neoplastic Langerhans cells

from reactive Langerhans cells (PMID: 28622183; PMID: 30124506). Compared to LCH, indeterminate dendritic cell tumor (IDCT) is extremely rare, resulting in paucity of literature on this entity. However, a recent multi-institutional study showed cyclinD1 positivity in IDCT when performed (PMID: 39361706- Supplemental table 1).

In ambiguous cases, where there are dense aggregates of CD1a-positive cells with variable cyclinD1 expression, I tend to be more conservative in my approach and call them as 'atypical dendritic cell proliferation' with subsequent NGS and a repeat biopsy if lesions remain persistent.

12. **Question: When do we do ALK IHC? In histiocytic lesions?**

Answer: In my practice, we perform next generation sequencing (both DNA and RNA sequencing) in all histiocytic neoplasms (RDD, JXG, ECD, LCH, MHN) as part of our Histiocytosis Clinic Protocol. Since RNA sequencing is performed in all my cases, I generally do not perform ALK immunostaining. However, in cases with limited tissue where NGS is more likely to fail, I perform ALK IHC (not D5F3 clone; I typically use the 5A4 or 1A4 antibody clones).

In general, in cases where the follow-up is questionable or pursuing NGS may not be feasible, it is recommended to do a minimum panel of IHC: CD68/CD163, factor 13a, S100, cyclinD1, CD1a, ALK and pan-TRK. A langerin IHC can be pursued if CD1a is positive.

13. **Question: What is your experience with IRF8 by IHC for myeloid sarcomas with monocytic differentiation?**

Answer: I do not have any experience with IRF8 immunostaining in myeloid sarcomas. Based on a prior study (PMID: 39569507), it is my understanding that IRF8 can be positive in histiocytic neoplasms and therefore may not be entirely reliable to distinguish myeloid sarcomas with monocytic differentiation from histiocytic neoplasms (specifically MHNs).

14. **Question: Can langerin be positive in reactive conditions?**

Answer: Yes, langerin can be positive in reactive conditions and they are co-expressed in CD1a-positive cells. Examples include dermatopathic lymphadenitis (PMID: 30147924),

cutaneous inflammatory dermatosis (PMID: 28622183), and pulmonary diseases (PMID: 36160158).

Langerin expression without CD1a co-expression is present in littoral cell angiomas as well as hepatic sinusoids and lymph node sinuses (refer to slide#40)[reference PMID: 15630529; PMID: 30130631].

15. **Question: How much trust you place in BRAF IHC vs BRAF pcr? We tend to do IHC more frequently for logistic ease.**

Answer: I do not trust BRAF VE1 IHC in my current practice; as I have observed both false positives (weak cytoplasmic blush; reference PMID: 40019725) and false negatives. When BRAF VE1 IHC is positive, I always reflex to NGS (comprehensive NGS or ddPCR for BRAF V600E mutation) to confirm the presence of genetic mutation.

The utility of BRAF VE1 immunostaining should be interpreted within the context of each institution laboratory validation protocols to determine whether the IHC result is sufficiently reliable to serve as a standalone test.

16. **Question: Most of the histiocytic proliferation can occur in multisystem fashion which mimic clinically malignant lesions. How do we actually define what makes it a benign or malignant entity, apart from overt morphology of pleomorphism and mitosis?**

Answer: Defining whether a histiocytic lesion is 'benign' or 'malignant' extends beyond histopathology and instead relies on integrating clinical behavior, extent of organ involvement (including presence/absence of risk organs such as liver, spleen and bone marrow), and genetic alterations (PMID: 41178439). Despite numerous advancements, I do believe there is limited comprehension of natural history and long-term outcomes in histiocytic disorders, necessitating the need for collaborative studies with longitudinal follow-up of larger patient cohorts.

17. **Question: How often you seen LCH in siblings? With BRAF mutation positive?**

Answer: In my histiocytosis practice, I have not encountered such a scenario. Therefore, I do not know the true incidence of LCH in siblings.

18. **Question: Do you encounter cyclinD1 positivity in ECD in cases without proved mutations?**

Answer: Yes, we do. While a majority of ECD cases usually harbor a pathogenic alteration involving the MAPK-ERK or PI3k-Akt pathway, approximately 5-10% of cases do not show any pathogenic variants. In our recent study, we showed 3 ECD cases with no genetic alterations being cyclinD1-positive [reference PMID: 41495601].

On the contrary, we can also see cyclinD1-negativity in a minor subset of ECD (~10%). In such cases, clinical presentation and pathognomonic radiologic findings (as shown in slide #27) suggestive of ECD should prompt comprehensive NGS analysis including DNA and RNA sequencing. In extremely rare instances, both cyclinD1 and NGS can be negative, and the diagnosis relies on clinical and radiologic correlation with possible repeat biopsies and repeat NGS testing.

19. **Question: What's your approach for sign out regarding cases with mixed histiocytic/dendritic components? Do you have a cutoff for CD1a % to call LCH/LCS vs other histiocytic categories?**

Answer: Mixed histiocytosis can present on the same site or different sites at the same time or different points in time.

i) I have seen two cases of LCH with RDD present at the same anatomic site as a collision tumor → in such settings, I would frame the diagnosis as 'Histiocytic Neoplasm; Subtype: Mixed Histiocytosis with features of LCH (xx% of the specimen) and RDD (xx% of the specimen)

ii) When it presents at different sites, then mixed histiocytosis becomes more of a clinical diagnosis – some cases may present years apart and the diagnosing pathologist may not have all relevant clinical information at the time of biopsy review.

There is no defined cut-off of CD1a% in LCH or LCS. In LCH, the lesional histiocytes are diffusely positive for CD1a (typically >50% tumor cells); whereas in MHN-LCS subtype, CD1a can be variable (<5% to >50% of tumor cells). MHN-LCS subtype can also show overlapping features with histiocytic sarcoma (HS) or interdigitating dendritic cell sarcoma (IDCS) subtypes (reference PMID: 37406859- figure 5); the key take away point in such cases is to note the

diagnosis as ‘malignant histiocytic neoplasm (MHNs)’ and indicate in the comment section that this has features of both LCS and HS/IDCS by immunophenotyping.

20. **Question: Have you encountered non-specific staining of CD68, which clone do you use?**

    Answer: Yes, we can observe non-specific staining of CD68, and we can see these in non-hematopoietic tumors, such as melanoma with the KP-1 antibody clone. Hence, there is always a need to use >1 histiocytic-lineage marker (CD68, CD163, CD4, PU.1) to confirm the histiocytic lineage. I prefer to use the CD68-PGM-1 antibody clone in practice, as this is a more specific histiocytic marker compared to other CD68 antibody clones.