A 74-year-old male presented with rectal pain; workup uncovered an anal mass, and a diagnosis of melanoma was rendered via histologic examination and immunohistochemical (IHC) studies. Droplet digital PCR (ddPCR)–based BRAF testing was performed and revealed the presence of BRAF-V600E, which is a common targetable genetic alteration in melanoma. Interestingly, the ratio of mutant to wild-type copy number was low (0.3%), whereas tumor cell percentage on tissue slides was 90%. With additional workup, BRAF-V600E IHC confirmed a very small subset of BRAF-V600E–positive cells, and a next-generation sequencing (NGS) panel revealed a pathogenic KIT variant, p.L576P, with an allele frequency of 63%. It was initially hypothesized that the main driver of the melanoma was the KIT alteration, whereas a small subclone (not detected by NGS, which has a 5% limit of detection) was driven by the BRAF-V600E detected by ddPCR. To determine whether there were morphologic differences between the 2 clones, a careful review of the histology was performed and revealed distinct morphology of the BRAF-V600E–positive cells, including pale cytoplasm, nuclear grooves, and infiltrating eosinophils. Additional IHC workup of the BRAF-V600E–positive cells showed coexpression of CD1a, Langerin, and S100, diagnostic of Langerhans cell histiocytosis (LCH). This diagnosis was unexpected and would have been missed without highly sensitive molecular testing; yet it is of clinical importance for the patient. This case raises important questions regarding the relationship between melanoma and LCH; moreover, it highlights the importance of integrating quantitative information in molecular data interpretation.

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Case Report
A 74-year-old male presented with rectal pain; workup revealed an anal mass, and histologic examination and immunohistochemical studies rendered a diagnosis of melanoma (whether it was primary cutaneous or mucosal was uncertain, because the lesion involved both aspects of the anus). A PET/CT scan was negative for local or satellite lesions or distant or nodal metastatic disease (clinical stage: N0M0). The patient underwent resection, followed by molecular testing to guide treatment and prognosis. Droplet digital PCR (ddPCR)–based BRAF-V600E/K testing was then performed at Mayo Clinic. Preanalytically, tissue slides were reviewed and tumor cell percentage was estimated to be >90%. The whole tumor tissue on the slides was macrodissected and submitted for testing. A BRAF-V600E alteration, which is the most common targetable alteration for melanoma, was identified. Interestingly, the ratio of mutant to wild-type copy number was low compared with the tumor percent (0.3% vs >90%, respectively; Figure 1). This discrepancy prompted us to investigate further.

We first performed BRAF-V600E immunohistochemistry (IHC; monoclonal mouse, clone VE1, IgG2a, Abcam) to confirm the presence of this variant. Indeed, BRAF-V600E IHC stained a small subset of cells (Figure 2), confirming the ddPCR result. This finding was initially thought to be the rare presence of a subclone harboring BRAF-V600E within the melanoma, with most of the remaining tumor cells (BRAF-V600E–negative) having a different driver alteration. To test this hypothesis, we then performed a melanoma next-generation sequencing (NGS) panel (BRAF, GNAQ, GNA11, KIT, NRAS) to assess for the presence of an alternative driver alteration. Indeed, we found a KIT alteration, c.1727T>C (exon 11), p.L576P with a variant allele frequency of 63.2%, well-matched with the tumor percent. The limit of detection for this melanoma NGS panel is 5%, thus the low level of the BRAF-V600E variant was not detected. At this point, we suspected that the main component of the melanoma was driven by the KIT alteration, whereas a small subclone was driven by the BRAF variant. From a clinical standpoint, detection of a subclonal BRAF-V600E has uncertain significance in terms of therapeutic management,
because ideally a targetable variant should be clonal rather than subclonal.

Finally, we wanted to see whether the subclone harboring the \textit{BRAF} V600E was morphologically distinct from the main melanoma population driven by \textit{KIT} L576P. We carefully reviewed the histology of the tumor, which revealed that the \textit{BRAF} V600E–positive cells were in fact smaller and characterized by grooved/folded nuclei with inconspicuous nucleoli and abundant pale eosinophilic cytoplasm (Figure 2). In addition, increased eosinophils were intermixed within these cells. All of these morphologic features were suggestive of a diagnosis of Langerhans cells histiocytosis (LCH). Additional IHC stains were then ordered and showed that the \textit{BRAF} V600E–positive cells were also strongly expressing CD1a (monoclonal mouse, clone NCL-L-CD1a-235, IgG1 kappa; Leica), Langerin (monoclonal mouse, clone NCL-L-Langerin, IgG2b kappa; Leica), and S100 (polyclonal rabbit, clone NCL-L-S100p, Leica), while negative for Melan A (monoclonal mouse, clone A103, IgG1 kappa; Dako), CD163 (monoclonal mouse, clone NCL-L-CD163, IgG1; Leica), and c-kit (monoclonal rabbit, clone YR145, IgG; Cell Marque) (Figure 3). Notably, the melanoma cells, which show pleomorphic nuclei with distinct nucleoli and abundant eosinophilic cytoplasm (Figure 2), were expressing Melan A, c-kit, and weak S100 (Figure 3).
IHC pattern supported the diagnosis of focal LCH coexisting with melanoma.

Separate microdissection of the LCH foci and the melanoma component was considered for running separate molecular testing and better characterizing the genetic profile of each entity in its purity, as well as analyzing shared alterations and pathways activation. However, LCH foci were too small and ill-defined to allow for such analysis. Further studies are warranted to investigate the possible relation between LCH and melanoma when occurring together, with or without a shared BRAF pathogenic alteration.

After surgery, the patient was treated with local radiotherapy. The oncology team planned on treating him with adjuvant immunotherapy, anti–PD-1, for 1 year. However, a follow-up PET/CT scan performed approximately 8 months after the surgery showed new left inguinal lymphadenopathy, suspicious for metastatic disease. A lymphadenectomy

**Figure 2.** High magnification (original magnification ×20) of a small group of BRAF V600E–positive cells. (A) On H&E staining, the cells in the circle have grooved/folded nuclei with inconspicuous nucleoli, abundant pale eosinophilic cytoplasm, and eosinophils are intermixed. The melanoma cells outside the circle have pleomorphic nuclei with distinct nucleoli and abundant eosinophilic cytoplasm. (B) The cells in the circle strongly stain with BRAF V600E IHC, cytoplasmic stain. Abbreviations: H&E, hematoxylin-eosin; IHC, immunohistochemistry; LCH, Langerhans cell histiocytosis.

**Figure 3.** High magnification (original magnification ×20) of the additional immunostains. The BRAF V600E–positive cells (LCH cells) are negative for Melan A, c-kit, and CD163, and positive for Langerin, CD1a, and S100. The surrounding melanoma cells are positive for Melan A, c-kit, and weaker S100. CD163 marks admixed reactive macrophages. Abbreviation: LCH, Langerhans cell histiocytosis.
was performed at a different institution, and confirmed many lymph nodes positive only for metastatic melanoma, without morphologic evidence of LCH mentioned in the clinical notes available. However, \(\text{BRAF} V600E\) testing, using a sensitive method like ddPCR, was not performed at the outside institution to rule out a low-level LCH involvement. Alternative immunotherapies for treating the melanoma are now being considered by the clinical team, and the patient is being monitored for the presence and extent of LCH.

**Discussion**

High sensitivity is often a desirable feature in molecular diagnostic tools, allowing for accurate diagnosis and high negative predictive value. However, integrating quantitative information and other clinical and laboratory findings is crucial for accurate interpretation of test results. This report presents a case of anal melanoma with a \(\text{BRAF} V600E\) variant detected by ddPCR. What was striking about the \(\text{BRAF} V600E\) alteration was the low-level signal (0.3%, mutant/wild-type copy number ratio) compared with the estimated melanoma percentage. The discrepancy between the tumor cell percentage and the signal of a driver alteration prompted careful review of the histology, which in turn helped identify an unexpected coexisting disease process. A valuable teaching point from this case is that, in molecular testing, integrating quantitative information in data interpretation is important. The variant allele frequency of a pathogenic alteration can shed light on the clonal architecture of a tumor and can help in confirming a clonal heterogeneity that might not be noticeable on histology or clinically.

Langerhans cell histiocytosis is a heterogeneous disease with diverse clinical manifestations and outcomes.\(^1\) Common genetic alterations involve the MAPK/ERK pathway, with \(\text{BRAF} V600E\) occurring in approximately 50% of cases.\(^3^{–}4\) Cases of LCH co-occurring with malignancies, including cutaneous melanoma, have been previously reported (Table 1),\(^5^{–}9\) with one case describing similar findings to ours, in which a cutaneous melanoma was associated with a very small focus of adjacent/intermixed LCH.\(^9\) Notably, a few cases of LCH associated with nonmalignant melanocytic lesions have also been previously reported in children (Table 1).\(^10^{,}11\) In the current case, which would be the first to describe a case of LCH associated with mucosal melanoma, the amount of LCH was minimal and objectively challenging to detect with histologic evaluation. The ability to detect LCH and explain apparently discordant molecular findings had important implications for our patient, particularly for the interpretation of the \(\text{BRAF} V600E\) variant.

Adult LCH can be a localized or a multisystem disease, and its treatment and prognosis depend on the organ system involved.\(^1^{,}12\) For instance, nonpulmonary, unifocal LCH in adults tends to have an excellent prognosis, with a 5-year overall survival of 94%; however, recurrence is possible and can occur in a different organ system;\(^4\) thus, clinical follow-up is extremely important. Of note, studies have suggested that \(\text{BRAF} V600E\)-mutated LCH can be effectively treated with \(\text{BRAF}\) inhibitors in frontline and relapse settings.\(^1^{,}13\) It is important for clinicians to be aware of coexisting malignancies with LCH, because it can affect clinical management and disease monitoring. Overall, the decision to treat LCH when there is a coexisting malignancy may depend on different factors, including the LCH subtype (unifocal, single-system pulmonary, or multifocal and multisystem), the presence of a targetable \(\text{BRAF}\) alteration, the type and extent of the concurrent malignancy, and the patient’s baseline clinical performance.\(^1\) From the perspective of treating the melanoma, knowing that LCH, but not the metastatic melanoma, harbors \(\text{BRAF} V600E\)...

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**Table 1. Cases of LCH Associated With Melanocytic Skin Lesions**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Findings</th>
<th>PubMed ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Melanomas</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richmond et al(^5)</td>
<td>1995</td>
<td>Intranal LCH + cutaneous melanoma</td>
<td>7607630</td>
</tr>
<tr>
<td>Roufosse et al(^6)</td>
<td>1998</td>
<td>Intranal LCH + lymphocyte predominance Hodgkin disease + cutaneous melanoma</td>
<td>9490285</td>
</tr>
<tr>
<td>Tirilomis et al(^7)</td>
<td>2002</td>
<td>Pulmonary LCH + metastatic melanoma</td>
<td>12362286</td>
</tr>
<tr>
<td>Ma et al(^8)</td>
<td>2019</td>
<td>Tissue (?) LCH + melanoma (cutaneous?) (2 cases)</td>
<td>30597769</td>
</tr>
<tr>
<td>Goto et al(^9)</td>
<td>2022</td>
<td>Cutaneous LCH + cutaneous melanoma, both (\text{BRAF} V600E)-positive</td>
<td>34792818</td>
</tr>
<tr>
<td>Buglioni et al</td>
<td>2023</td>
<td>LCH + mucosal melanoma</td>
<td>Current report</td>
</tr>
<tr>
<td><strong>Nevi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Berk and Lane(^10)</td>
<td>2010</td>
<td>LCH + agminated Spitz nevi</td>
<td>20609146</td>
</tr>
<tr>
<td>Surinach et al(^11)</td>
<td>2013</td>
<td>LCH + eruptive nevi</td>
<td>23677112</td>
</tr>
</tbody>
</table>

Abbreviation: LCH, Langerhans cell histiocytosis.
is also crucial. Finally, detection of the “real” driver alteration KIT L576P favors this anal melanoma being mucosal in origin (over cutaneous), helping to better define this lesion and further optimize treatment.14

Conclusions
The current case highlights the importance of carefully analyzing and correlating histologic, molecular, and clinical findings. Highly sensitive molecular methods, such as ddPCR, are a powerful tool to detect potentially targetable pathogenic alterations and are fundamental for personalized patient care. From a laboratory perspective, in addition to assessing for the presence of a pathogenic variant, it becomes important to quantify that variant in order to help better characterize and understand the biology of the disease process being tested. From a clinical perspective, noting the variant and its quantification/frequency will help optimize patient management, which allows for focusing on the right target; administering a drug that is not needed might be as harmful as not giving an effective drug.

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