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BRAF V600E mutations are not an oncogenic driver of solitary xanthogranuloma and reticulohistiocytoma: Testing may be useful in screening for Erdheim-Chester disease

Brian S. Hoyt[a,c], Shaofeng Yan[a,c], Konstantinos D. Linos[a,c], Shabnam Momtahen[a,c], Aravindhan Sriharan[a,c], Tien-Anh N. Tran[b], Gregory J. Tsongalis[a,c], Rebecca R. O'Meara[a,c], Donald C. Green[a,c], Robert E. LeBlanc[a,c,*]

[a] Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, Lebanon, NH, USA
[b] Department of Pathology, Advent Health Orlando, FL, USA
[c] Audrey and Theodor Geisel School of Medicine at Dartmouth, Hanover, NH, USA

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ABSTRACT

BRAF V600E is the predominant oncogenic driver of L-group histiocytoses, which includes Erdheim-Chester disease (ECD); however, limited data exist on the prevalence of this mutation in sporadic XG family lesions. This study sought to determine the incidence of *BRAF* V600E mutation in a clinically annotated cohort of patients with xanthogranulomas (XG) and reticulohistiocytomas (RH). A retrospective review of 58 lesions was performed, including 41 XG and 17 RH. Immunohistochemistry (HC) and PCR-based methods were performed to evaluate for the *BRAF* V600E mutation. The *BRAF* V600E mutation was detected by IHC/PCR in 3 RH from an adult who had no history of arthritis, malignancy, xanthelasma, diabetes insipidus or bone pain. All other XG and RH were negative for the *BRAF* V600E mutation. No associated systemic diseases were identified in this cohort. Our findings suggest that *BRAF* V600E mutations are not an oncogenic driver of sporadic XG and solitary RH. Therefore, identification of such a mutation in a patient with multiple lesions should raise consideration for ECD. We also report the first known *BRAF* V600E mutation in a patient with multiple reticulohistiocytomas.

1. Introduction

Activating MAPK pathway mutations are responsible for the majority of L-group histiocytoses, a category encompassing Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD) (Emile et al., 2016; Ozkaya et al., 2018; Badalian-Very et al., 2010; Haroche et al., 2012). *BRAF* V600E activating mutations have been identified in approximately half of these patients. Patients with ECD can develop cutaneous lesions indistinguishable from benign, solitary xanthogranulomas (XG) in addition to xanthelasma-like lesions and rarer histiocyte-rich dermal and subcutaneous infiltrates. BRAF V600E immunohistochemistry and molecular testing have become increasingly available in pathology laboratories offering specificity for detection of the V600E mutation. In light of these advancements, the Histiocyte Society recommends molecular testing of extracutaneous and disseminated XG for gain-of-function mutations in *BRAF*, *NRAS*, *KRAS*, or *MAP2K1* to help exclude ECD (Emile et al., 2016). However, limited information exists regarding the prevalence of *BRAF* V600E mutations is XG family lesions that are not associated with ECD (Haroche et al., 2012; Go et al., 2014; Techavichit et al., 2017; Diamond et al., 2016; Chakraborty et al., 2014; Picarsic et al., 2018; Durham et al., 2018). There is also no previous literature discussing the prevalence of *BRAF* mutations in reticulohistiocytoma (RH), which is considered by some to be a variant of XG despite their often distinct histologic appearances and contrasting clinical implications (Emile et al., 2016; Go et al., 2014; Burgdorf and Zelger, 1996). A retrospective analysis of several dozen patients representing two institutions was undertaken in order to determine the prevalence of *BRAF* mutations in RH and XG.

2. Materials and methods

A search in our archives for diagnoses of XG and RH identified 54 biopsies between 1997 and 2018. Two additional RH biopsies were subsequently included in the study since they were taken from a patient already belonging to our cohort. Eight additional biopsies were contributed by a collaborating peer institution. Six biopsies were excluded

* Corresponding author at: Division of Dermatopathology, Department of Pathology and Laboratory Medicine, One Medical Center Dr., Lebanon, NH 03756, USA.
E-mail address: Robert.E.LeBlanc@hitchcock.org (R.E. LeBlanc).

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Table 1
Patient summary.

	Age, mean years (range)	Sex	BRAF positive IHC	BRAF positive PCR
Reticulohistiocytoma 15 patients	40 (8–65)	9 M 6F	1/15	1/15
Xanthogranuloma 40 patients	27 (< 1–67)	22 M 19F	0/41	0/41

Abbreviations: F – female, IHC – immunohistochemistry, M – male, PCR – polymerase chain reaction, y –years.

because there was inadequate material to perform immunohistochemistry, bringing the total to 58 biopsies from 56 patients (Table 1). All biopsies were reviewed by five board-certified dermatopathologists (RL, KL, SY, AS, and SM) for diagnostic confirmation. Consensus diagnosis was reached on 41 xanthogranulomas (XG) and 17 reticulohistiocytomas (RH) since XG family lesions generally contain variable proportions of xanthomatous histiocytes and Touton giant cells characteristic of XG as well as cells with ground glass cytoplasm and mild nuclear pleomorphism that support a diagnosis of RH. In these cases, a majority diagnosis prevailed. Two of the patients had multiple lesions. The remaining patients had solitary lesions.

BRAF V600E IHC (Abcam cat no: ab228461, clone: VE1) was performed on all 58 biopsies using the Bond-Rx (Leica-Microsystems, Buffalo Grove, IL. IHC slides cut at 4 μm and air dried at room temperature, staining was performed on the Leica Bond Rx automated stainer. Slides were backed for 30 min, dewaxed with Bond dewax solution (Cat # AR9222 Antigen Retrieval using Bond Epitope Retrieval 2 (Cat #AR9640), pH 9.0 solution 20 min at 100 deg. Primary antibody binding was visualized using Bond polymer Refine detection Kit (#DS9800) with Dab chromogen and hematoxylin counterstain.

In addition, PCR testing was performed on all 58 biopsies using the Idylla™ system BRAF/NRAS cartridge (Biocartis, Mechelen, Belgium). This is a fully integrated, cartridge-based platform that provides automated sample processing (deparaffinization, tissue digestion and DNA

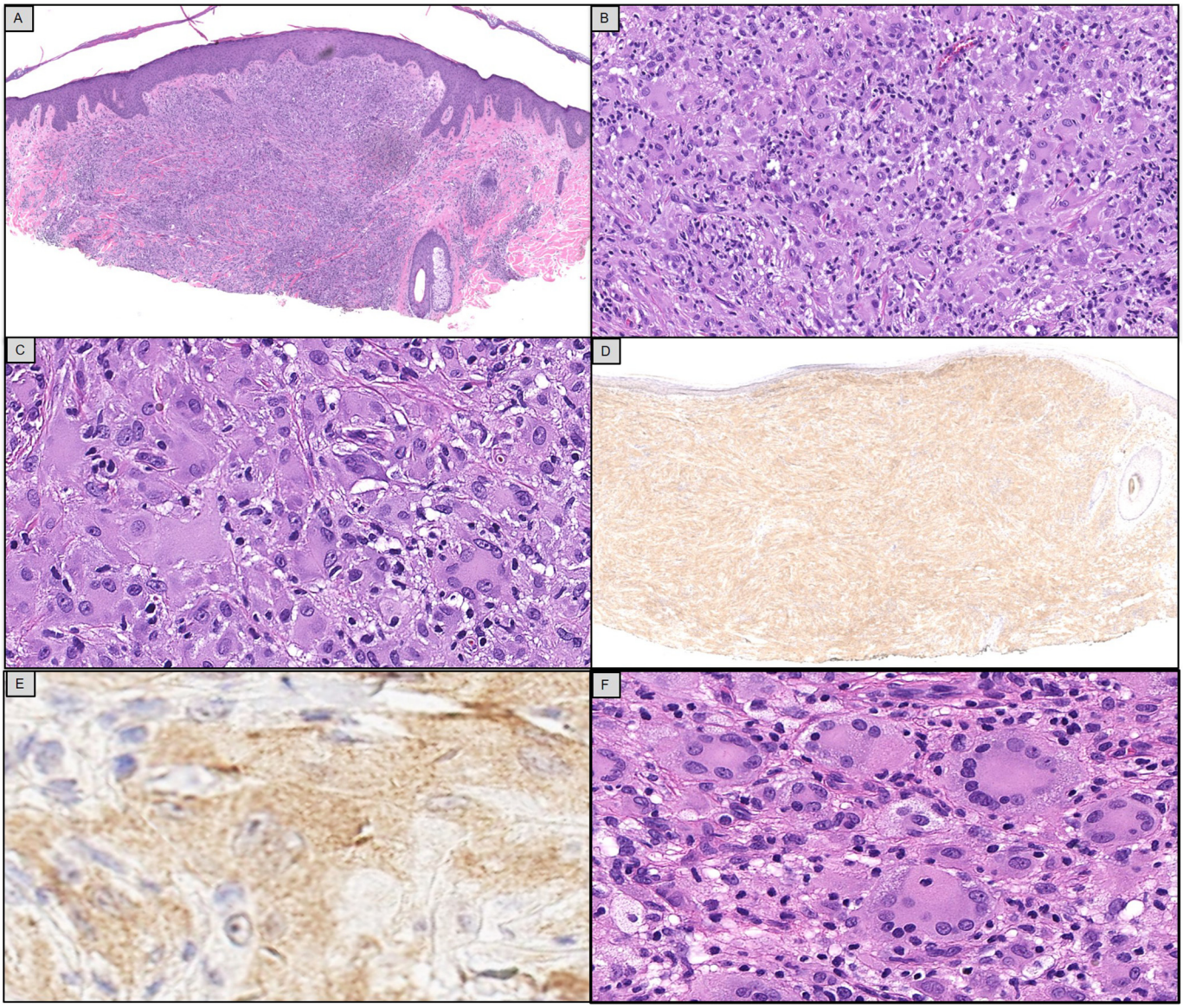

Fig. 1. Reticulohistiocytoma from the left knee of a 57-year-old man. The vaguely fascicular organization of large, epithelioid histiocytes is punctuated by foci of mixed inflammation (A. H&E, x40). Lesional cells are dyshesive and possess abundant, glassy amphoteric cytoplasm, ovoid nuclei with mild hyperchromasia, and prominent nucleoli (B and C. H&E, x200 and x400, respectively). The cells stained uniformly with *BRAF* IHC (D, x100; E, x400). In contrast, XG possessed Touton giant cells comprised of multinucleated histiocytes with lipidized wreaths.

extraction) and real-time PCR-based mutation detection with all reagents included in a single-use cartridge. The limit of detection is 5% allelic fraction.

3. Results

39 patients with XG, 14 patients with RH, and 1 patient with both XG and RH were included. Two patients had multiple lesions: a 57 year-old man with multiple RH and a 28 year-old woman with 3 XG family lesions. The remaining patients had solitary lesions. The patients with XG tended to be younger (mean age 27 years, range 3 months to 67 years) and included a greater proportion of pediatric patients (17 were under age 18). By comparison, RH patients were mostly adults (mean age 40 years, range 8 to 65 years) with only two pediatric patients, ages 12 and 17. None of the patients with either diagnosis had a history of an L-type histiocytosis, multicentric reticulohistiocytosis, neurofibromatosis, or relevant co-morbidities that would raise clinical suspicion for systemic illness such as xanthelasma, bone pain, arthralgia, diabetes insipidus or neurologic manifestations affecting gait or coordination. The median follow-up time for patients with RH was 13 years (range 2 to 21 years). The median follow-up time for patients with XG was 5 years (range 1 to 22 years).

The *BRAF* V600E mutation was detected by IHC and confirmed by PCR in a solitary patient with multiple reticulohistiocytomas. The 57-year-old otherwise healthy man had three RH located on his left knee, left forearm, and left groin. All had arisen over the course of a year. He had no history of arthritis, other rheumatologic illness, or malignancy. Over a fourteen-month follow-up period, he did not develop any additional RH. The patient has not undergone the requisite imaging studies to exclude ECD; however, he has no xanthelasma-like lesions, bone pain, history of diabetes insipidus or other symptoms of either cardiovascular or central nervous system involvement. The cells comprising all of his lesions had a CD68-positive S100-negative phenotype and expressed *BRAF* by immunohistochemistry [Fig. 1].

All other lesions in this investigation were negative for the *BRAF* mutation by both IHC and PCR [Table 1]. This included the 14 remaining RH, most of which were solitary lesions. Only one additional patient had multiple lesions. A 28-year-old woman had three lesions with varied histomorphologies including 2 XG and 1 RH by group consensus. This patient had no history of rheumatoid arthritis, malignancy or evidence of systemic disease at 6 years of follow-up.

4. Discussion

While the *BRAF* V600E mutation is present in approximately half of patients with ECD, our findings indicate that *BRAF* V600E is not an oncogenic driver of solitary skin-limited lesions, which is in keeping with the sparse data previously published on this topic (Haroche et al., 2012; Go et al., 2014). Altogether, the findings of these investigations suggest that BRAF immunohistochemistry and PCR testing could be a helpful adjunct in screening for ECD in a patient with multiple lesions or features otherwise clinically concerning for histiocytosis.

We have also documented the first known somatic *BRAF* mutation in RH, involving multiple lesions in a single patient. This patient lacked additional clinical features to establish a diagnosis of multicentric reticulohistiocytosis. While the disease has remained skin-limited, the possibility of ECD cannot be excluded given the short time to follow-up, the rapid development and the broad distribution of the lesions. Although the skin lesions of ECD are classically described as XG and not RH, the Histiocyte Society posits “Solitary reticulohistiocytoma (SRH) is simply a xanthogranuloma in which oncocytic macrophages and ground glass giant cells dominate (Emile et al., 2016).” ECD has been associated with additional cutaneous manifestations including xanthelasma-like lesions (XLL), histiocyte-rich proliferations resembling granuloma annulare, and subcutaneous nodules expanding the subcutaneous fibroadipose tissue and septa.(Chasset et al., 2016; Kobic et al., 2019) The appearance of multiple RH in an adult patient would raise clinical consideration for multicentric reticulohistiocytosis; however, ECD should also be given consideration, particularly when patients do not have a history of arthritis. XG family lesions are known to show histologic overlap. Our data suggest that the *BRAF* V600E mutation is neither an oncogenic driver of solitary RH nor XG.

A limitation of this investigation is that it did not assess for other MAPK pathway mutations beyond *BRAF V600E* that have also been implicated in ECD (Emile et al., 2016; Ozkaya et al., 2018; Haroche et al., 2012). BRAF was chosen since it is the most prevalent mutation and immunohistochemistry has become a widely available and accepted surrogate marker for the mutation in melanoma and thyroid carcinoma (Ritterhouse and Barletta, 2015; Mehes et al., 2014; Ballester et al., 2018). Additional data will be necessary to determine a sensitivity and specificity of IHC for detecting the *BRAF* V600E mutation in histiocytoses, as there is evidence that the proportion of BRAF-positive histiocytes staining with the antibody varies between cases of histiocytoses.(Melloul et al., 2019) However, all cases in our cohort were tested both by IHC and PCR with fully concordant results. Other mutations involving the MAPK pathway (e.g., *MAP2K1, ARAF, NRAS, KRAS,* and *ALK*) and a rare non-MAPK pathway mutation (*PIK3CA*) have been identified in ECD with decreasing frequency (Ozkaya et al., 2018; Brown et al., 2014; Emile et al., 2014; Heritier et al., 2015). The Histiocyte Society recommends molecular analysis for *BRAF* V600E and *MAP2K1* mutations when evaluating patients with widely disseminated cutaneous lesions or extracutaneous lesions suspicious for LCH and ECD;(Emile et al., 2016; Abla and Weitzman, 2015) however, a majority of dermatopathology laboratories do not routinely assess for *MAP2K1* mutations, in contrast with *BRAF* gene mutation assessment which is performed commonly in the care of patients with metastatic melanoma. As molecular laboratory technologies become increasingly available and affordable, additional investigations will be necessary in order to establish the prevalence of all ECD-associated mutations in sporadic XG and RH. This information will be critical for developing an adequate screening algorithm that could allow pathologists to establish earlier diagnoses. This could be particularly important when skin manifestations of ECD presage extracutaneous dissemination.

Lastly, *BRAF* mutations have been reported in other rare histiocytic disorders, including three cases of extracutaneous juvenile xanthogranuloma (Techavichit et al., 2017) and three cases of Rosai-Dorfman disease (RDD) that includes a recently reported case of combined RDD and LCC which responded to *BRAF* inhibitor therapy (Fatobene et al., 2018; Richardson et al., 2018; Mastropolo et al., 2019). Additionally, *BRAF* V600E mutations have been reported in histiocytic sarcomas (Go et al., 2014). Cutaneous XG harboring a *BRAF* V600E mutation was reported in a 13-year-old boy. Unlike patients in our cohort, however, he had an antecedent diagnosis of LCH (Bellinato et al., 2019). Rarer mutations involving the MAPK pathway, including *MAPK1*[26], *ARAF*, and *NRAS*[7], have indeed been described in xanthogranuloma. The PCR-based assay used in this study also assessed for mutations in *NRAS*, and none were identified in our cohort (see Supplementary Table 1 for the complete list of *BRAF* and *NRAS* mutations detectable by the assay). These observations underscore the need for more data in order to determine the degree of molecular overlap between XG family lesions associated with ECD and those that are not.

In conclusion, the indications for molecular analysis in the diagnosis of histiocytic neoplasms deserve critical evaluation as there are no formal guidelines for appropriate ancillary testing (Emile et al., 2016). Our data and antecedent literature suggest that solitary XG family lesions, including RH, do not harbor the *BRAF* V600E mutations. We also report the first somatic *BRAF* V600E mutation identified in an adult patient with multiple cutaneous RH. Each of his three lesions tested positive for the mutation. Although this patient is not clinically suspected of having ECD or multicentric reticulohistiocytosis, no full-body imaging has been performed and time to follow-up is limited. In summary, our observations suggest that the V600E mutation is neither an

oncogenic driver of sporadic XG nor RH. The identification of a *BRAF* V600E mutation in a patient with multiple non-Langerhans cell histiocyte-rich lesions should prompt clinical consideration for ECD.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yexmp.2019.104320.

Disclosures

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References

Abla, O., Weitzman, S., 2015. Treatment of Langerhans cell histiocytosis: role of BRAF/MAPK inhibition. Hematology Am Soc Hematol Educ Program. 2015, 565–570.
Badalian-Very, G., Vergilio, J.A., Degar, B.A., et al., 2010. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood 116 (11), 1919–1923.
Ballester, L.Y., Cantu, M.D., Lim, K.P.H., et al., 2018. The use of BRAF V600E mutation-specific immunohistochemistry in pediatric Langerhans cell histiocytosis. Hematol. Oncol. 36 (1), 307–315.
Bellinato, F., Maurelli, M., Colato, C., Balter, R., Girolomoni, G., Schena, D., 2019. BRAF V600E expression in juvenile xanthogranuloma occurring after Langerhans cell histiocytosis. Br. J. Dermatol. 180 (4), 933–934.
Brown, N.A., Furtado, L.V., Betz, B.L., et al., 2014. High prevalence of somatic MAP2K1 mutations in BRAF V600E-negative Langerhans cell histiocytosis. Blood 124 (10), 1655–1658.
Burgdorf, W.H., Zelger, B., 1996. The non-Langerhans' cell histiocytoses in childhood. Cutis 58 (3), 201–207.
Chakraborty, R., Hampton, O.A., Shen, X., et al., 2014. Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. Blood 124 (19), 3007–3015.
Chasset, F., Barete, S., Charlotte, F., et al., 2016. Cutaneous manifestations of Erdheim-Chester disease (ECD): clinical, pathological, and molecular features in a monocentric series of 40 patients. J. Am. Acad. Dermatol. 74 (3), 513–520.
Diamond, E.L., Durham, B.H., Haroche, J., et al., 2016. Diverse and targetable kinase alterations drive histiocytic neoplasms. Cancer Discov. 6 (2), 154–165.
Durham, B.H.L.-R.E., Abramson, D.H., Picarsic, J., Pastore, A., Mandelker, D., Walsh, M.F., Arcila, M.E., Ladanyi, M., Solit, D., Berger, M.F., Hyman, D.M., Ki, M., Dunkel, I., Geissman, F., Diamond, E.L., Abdel-Wahab, 2018. Activating mutations in CSF1R and additional receptor tyrosine kinases in sporadic and familial histiocytic neoplasms. Blood 132, 49.
Emile, J.F., Diamond, E.L., Helias-Rodzewicz, Z., et al., 2014. Recurrent RAS and PIK3CA mutations in Erdheim-Chester disease. Blood 124 (19), 3016–3019.
Emile, J.F., Abla, O., Fraitag, S., et al., 2016. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. Blood 127 (22), 2672–2681.
Fatobene, G., Haroche, J., Helias-Rodzwicz, Z., et al., 2018. BRAF V600E mutation detected in a case of Rosai-Dorfman disease. Haematologica 103 (8), e377–e379.
Go, H., Jeon, Y.K., Huh, J., et al., 2014. Frequent detection of BRAF(V600E) mutations in histiocytic and dendritic cell neoplasms. Histopathology 65 (2), 261–272.
Haroche, J., Charlotte, F., Arnaud, L., et al., 2012. High prevalence of BRAF V600E mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses. Blood 120 (13), 2700–2703.
Heritier, S., Saffroy, R., Radosevic-Robin, N., et al., 2015. Common cancer-associated PIK3CA activating mutations rarely occur in Langerhans cell histiocytosis. Blood 125 (15), 2448–2449.
Kobic, A., Shah, K.K., Schmitt, A.R., et al., 2019. Erdheim-Chester disease: expanding the spectrum of cutaneous manifestations. Br. J. Dermatol.
Mastropolo, R., Close, A., Allen, S.W., McClain, K.L., Maurer, S., Picarsic, J., 2019. BRAF-V600E-mutated Rosai-Dorfman-Destombes disease and Langerhans cell histiocytosis with response to BRAF inhibitor. Blood Adv. 3 (12), 1848–1853.
Mehes, G., Irsai, G., Bedekovics, J., et al., 2014. Activating BRAF V600E mutation in aggressive pediatric Langerhans cell histiocytosis: demonstration by allele-specific PCR/direct sequencing and immunohistochemistry. Am. J. Surg. Pathol. 38 (12), 1644–1648.
Melloul, S., Helias-Rodzewicz, Z., Cohen-Aubart, F., et al., 2019. Highly sensitive methods are required to detect mutations in histiocytoses. Haematologica 104 (3), e97–e99.
Ozkaya, N., Rosenblum, M.K., Durham, B.H., et al., 2018. The histopathology of Erdheim-Chester disease: a comprehensive review of a molecularly characterized cohort. Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc. 31 (4), 581–597.
Picarsic, J.A.R., Jaffe, R., Diamond, E.L., Durham, B.H., Abdel-Wahab, A., 2018. Genomic analysis of C-group juvenile Xanthogranuloma family lesions identifies novel kinase alterations and distinctive morphologic patterns. Pediatr. Blood Cancer 66, e27548.
Richardson, T.E., Wachsmann, M., Oliver, D., et al., 2018. BRAF mutation leading to central nervous system rosai-dorfman disease. Ann. Neurol. 84 (1), 147–152.
Ritterhouse, L.L., Barletta, J.A., 2015. BRAF V600E mutation-specific antibody: a review. Semin. Diagn. Pathol. 32 (5), 400–408.
Techavichit, P., Sosothikul, D., Chaichana, T., Teerapakpinyo, C., Thorner, P.S., Shuangshoti, S., 2017. BRAF V600E mutation in pediatric intracranial and cranial juvenile xanthogranuloma. Hum. Pathol. 69, 118–122.