Clinical and therapeutic implications of BRAF fusions in histiocytic disorders

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DEAR EDITOR,

Histiocytic disorders represent a collection of hematologic diseases with varied clinical presentations [1]. The identification of an oncogenic driver has enabled the classification of some of the histiocytic disorders as neoplasms [1]. The activation of the mitogen-activated protein kinase (MAPK)-extracellular-signal-regulated kinase (ERK) pathway is the hallmark of Erdheim-Chester disease (ECD) and Langerhans cell histiocytosis (LCH) [2]. BRAF\textsuperscript{V600E} mutations are identified in 50–60% of patients with LCH and ECD and represent the most conspicuous mechanism for ERK activation [2, 3]. Additionally, one-third of patients with Rosai-Dorfman disease (RDD) have mutations in the MAPK-ERK pathway [2]. While the MAPK-ERK pathway mutations are ubiquitous in histiocytic disorders, little is known about the prevalence, pathogenic and clinical significance of BRAF fusions. Limited data in the form of case reports suggest that BRAF fusions can serve as alternative mechanisms of ERK activation [3, 4, 5], but the implications of BRAF and MEK-inhibitor therapy in histiocytosis harboring BRAF fusions are unknown. We conducted this study to examine the frequency, clinical features, and treatment outcomes among patients with histiocytic disorders harboring BRAF fusions. We also summarized the published reports of BRAF fusions in histiocytic disorders.

After approval by the institutional review board, we screened all new patients with histiocytic disorders seen at our institution between 01/11/2016 and 06/30/2021. All cases with confirmed histopathologic diagnoses of LCH, ECD, RDD, adult xanthogranuloma (AXG), juvenile xanthogranuloma (JXG), histiocytic sarcoma (HS), and Langerhans cell sarcoma (LCS) were analyzed. Only those patients with adequate BRAF testing were included in the final study population. Adequate BRAF testing was defined as, (i) unequivocally positive for BRAF\textsuperscript{V600E} immunostain (clone: VE1, Abcam, Cambridge, MA) with or without molecular confirmation or (ii) successful multigene next-generation sequencing with RNA fusion analysis (mostly Tempus or FoundationOne); required if VE1 immunostain was equivocal or negative. We also performed an extensive literature review for reports of BRAF fusions among histiocytic disorders through the PubMed search engine by using keywords: “BRAF fusion”, “histiocytosis”, “histiocytic disorders”, “Langerhans cell histiocytosis”, “Erdheim-Chester disease”, “Rosai-Dorfman disease”, “xanthogranuloma”, “Langerhans cell sarcoma”, and “histiocytic sarcoma”. Immunostaining for phospho-ERK (p-ERK, clone: D13.14.4E, Cell Signaling, Danvers, MA) was performed on formalin-fixed paraffin-embedded tissue sections using standard immunohistochemical methods on automated staining platforms and reviewed by two pathologists (K.L.R. and A.R.).

Response assessment was defined based on the Consensus recommendations. One hundred and twenty-six patients with a diagnosis of histiocytic disorder and adequate BRAF testing were identified. BRAF fusions were detected in seven (6%) patients. The frequency of BRAF fusions according to disease subtypes in our cohort was as follows: AXG/JXG (4/7 [57%]), ECD (2/46 [4%]), LCH (1/41 [2%]), RDD (0/23 [0%]), and HS/LCS (0/9 [0%]). The median age at diagnosis for patients with BRAF fusion cases was 34 years (range, 7–81 years) and 5 (71%) were females. We also identified 16 cases of histiocytosis with BRAF fusions reported in the literature. The clinical and molecular characteristics from our cohort as well as the previous reports are shown in Table 1. In the combined cohort of 23 patients, the median age at diagnosis was 19 years (range, 0.5–81 years) and 60% were females. The distribution of BRAF fusions by disease subtypes was as follows: AXG/JXG (10/23, 43%), LCH (7, 30%), ECD (3, 13%), non-LCH not otherwise specified (2, 9%), and HS/LCS (1, 4%). Most of the patients (13, 56%) had a single-system disease. The skin was the most common site of involvement (11, 50%) followed by bone (7, 32%), brain (5, 23%), and lung (4, 18%).

We identified 17 different BRAF fusions with several being recurrent (RNF11-BRAF in 3, BICD2-BRAF in 3, PACSIN2-BRAF in 2, and MS4A6A-BRAF in 2; Table 1). In the Mayo Clinic cohort, the data on the breakpoints of the BRAF fusions were available for six patients. All six of these BRAF fusions had intact kinase domain regions, Fig. 1. Three patients (MC-4, MC-6, and MC-7) had adequate tissue available for p-ERK immunohistochemistry and demonstrated moderate to strong nuclear and cytoplasmic (2–3+) p-ERK expression (Supplementary Fig. 1).

In our cohort, two patients with ECD underwent treatment with a MEK inhibitor (cobimetinib). The first patient (MC-1) harbored UBTD2-BRAF fusion and completed 12 cycles (12 months) of cobimetinib resulting in partial response (PR) in the lesions of brain parenchyma and tibia, with a first response within 2 months after initiation of cobimetinib (Supplementary Fig. 1). She also received intra-arterial melphalan for the residual brain parenchymal lesion, resulting in further tumor shrinkage. She continued to be in a sustained PR at the last follow-up 2.5 years from diagnosis. The second patient (MC-2) who underwent cobimetinib treatment had RNF11-BRAF fusion and achieved PR in the perirenal soft tissue, vertebral lytic lesion, along with a resolution of the bilateral pleural effusions at 2 months. She developed intolerable adverse effects (fatigue, rash, diarrhea, fever, nausea, and vomiting) after two cycles of cobimetinib resulting in treatment discontinuation but remained in a sustained PR 6 months after drug discontinuation. From a literature review, one patient (LR-12) was treated with cobimetinib as a second-line treatment and achieved a complete response [6]. While point mutations in BRAF are well-described in ECD and LCH, data on BRAF fusions are limited. Our series represents the largest study to date focusing on patients with histiocytosis and BRAF

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fusions. The presence of a BRAF fusion was uncommon in our overall cohort (~5%), but quite common in AXG/JXG subgroup (>30%). BRAF fusions are also an uncommon occurrence in most other neoplasms. A previous report utilizing comprehensive genomic profiling of solid tumors identified the presence of a BRAF fusion in 55 out of 20,573 (0.3%) patients, most notably in melanomas and pilocytic astrocytomas [7]. Interestingly, certain neoplasms with ectodermal origins have a high proportion of kinase fusions including BRAF. The spitzoid tumors/spitzoid melanomas harbor kinase gene fusions (including ALK, BRAF, NTRK, and ROS1) in up to 50% of patients [8] while pilocytic astrocytomas demonstrate BRAF fusions in 25–40% of the cases [9]. Apart from the common ectodermal origin, other associations between these tumors are limited and it is difficult to postulate with confidence as to why the AXG/JXG patients are enriched in BRAF fusions. On comparing the fusion partners of BRAF between our cohort and previous reports in solid tumors, all except the AGAP3-BRAF were novel [7]. Similar to our findings, previous reports of BRAF fusions in melanocytic tumors and histiocytic disorders have also demonstrated intact BRAF kinase domains [4, 10].

Increased ERK phosphorylation has been demonstrated in melanoma cell lines with induced BRAF fusions further suggesting the functional potential of these fusions [10, 11]. In our cohort of seven patients, three had adequate tissues for p-ERK Immunohistochemistry. All three patients expressed p-ERK (2+ to 3+), further strengthening the hypothesis that these BRAF fusions cause downstream MAPK-ERK activation. Similarly, we have recently demonstrated in a case of CSF1R-mutated ECD that mutation outside of the MAPK pathway was associated with negative p-ERK immunohistochemistry, with no response to MEK-inhibitor therapy [12]. While the evidence of functionality of BRAF fusion is defined, there are limited data on the role of targeted therapy in patients harboring these fusions. The response to MEK inhibition in our study suggests that this may be an effective treatment strategy for patients harboring BRAF-fusions like other MAPK-ERK activated histiocytosis. Interestingly, RAF inhibition in patients with BRAF fusions may not be an effective strategy. A prior study of melanocytic tumors cells lines with BRAF fusions demonstrated a paradoxical RAS-independent MAPK activation upon treatment with first- and second-generation RAF inhibitors and this was attributed to the fusion partners for BRAF in these cell lines - FKBPI-15-BRAF and SKAP2-BRAF [13]. Additionally, the RNF11-BRAF fusion is noted to sensitize murine pro-B cell Ba/F3 cells to MEK inhibition, but not RAF inhibition by vemurafenib [14, 15]. It is unclear if a concomitant BRAF and MEK inhibition would lead to better outcomes in these patients and further studies are needed to determine the role of combination therapy.

In summary, we report a robust collation of cases with BRAF fusions in patients with histiocytic disorders. The presence of BRAF fusions was uncommon in our overall cohort (~5%), but quite common in AXG/JXG subgroup (>30%). BRAF fusions are also an uncommon occurrence in most other neoplasms. A previous report utilizing comprehensive genomic profiling of solid tumors identified the presence of a BRAF fusion in 55 out of 20,573 (0.3%) patients, most notably in melanomas and pilocytic astrocytomas [7]. Interestingly, certain neoplasms with ectodermal origins have a high proportion of kinase fusions including BRAF. The spitzoid tumors/spitzoid melanomas harbor kinase gene fusions (including ALK, BRAF, NTRK, and ROS1) in up to 50% of patients [8] while pilocytic astrocytomas demonstrate BRAF fusions in 25–40% of the cases [9]. Apart from the common ectodermal origin, other associations between these tumors are limited and it is difficult to postulate with confidence as to why the AXG/JXG patients are enriched in BRAF fusions. On comparing the fusion partners of BRAF between our cohort and previous reports in solid tumors, all except the AGAP3-BRAF were novel [7]. Similar to our findings, previous reports of BRAF fusions in melanocytic tumors and histiocytic disorders have also demonstrated intact BRAF kinase domains [4, 10].

### Table 1. Summary of clinical characteristics of patients with BRAF fusions in patients with histiocytic disorders.

| Cohort | Age (yrs)/sex | Type | BRAF fusion | Organ involvement | Frontline therapy | Response |
|--------|---------------|------|-------------|-------------------|-------------------|----------|
| MC-1   | 27/F          | ECD | UBTD2-BRAF  | Brain, bone       | Cobimetinib       | PR (sustained 12 months) |
| MC-2   | 32/F          | ECD | RNF11-BRAF  | Bone, kidney, heart, lung, sinus | Cobimetinib       | PR (sustained at 8 months) |
| MC-3   | 55/F          | LCH | LMTK2-BRAF  | Bone              | Radiation + zoledronic acid | PR |
| MC-4   | 81/M          | AXG | AGAP3-BRAF  | Skin, multicentric | Observation       | - |
| MC-5   | 34/F          | AXG | ARRB1-BRAF  | Skin, multicentric | Observation       | - |
| MC-6   | 60/F          | AXG | UBR2-BRAF   | Lung, sclera, skin (disseminated) | Observation       | - |
| MC-7   | 7/M           | JXG | FNB1P1-BRAF | Spinal cord       | Surgery + clofarabine | CR |
| LR-12  | 4/M           | LCH | BICD2-BRAF  | Bone              | NA                | - |
| LR-22  | 37/F          | LCH | CSF2RA-BRAF | Thyroid, node, salivary gland | NA                | - |
| LR-32  | 57/M          | LCH | PACSN2-BRAF | Node, oral mucosa | NA                | - |
| LR-42  | 29/F          | LCH | SPPL2A-BRAF | Skin              | NA                | - |
| LR-52  | 1/M           | JXG | RNF11-BRAF  | Skin              | NA                | - |
| LR-62  | 0.5/M         | JXG | MS4A6A-BRAF | Skin              | NA                | - |
| LR-72  | 14/M          | JXG | BICD2-BRAF  | Brain             | NA                | - |
| LR-82  | 12/F          | JXG | BICD2-BRAF  | Skin (disseminated), bone, node, lung | NA | - |
| LR-92  | 16/F          | HS/LCS | MTAP-BRAF | Subcutaneous | Surgery | CR |
| LR-103 | 12/F          | JXG | MS4A6A-BRAF | Lung, node, skin (disseminated) | Clofarabine | PR |
| LR-113 | 6/M           | LCH | PACSN2-BRAF | Bone, skin | Prednisone/vinblastine | SD |
| LR-125 | NA            | ECD | PICALM-BRAF | Bone, brain | Vinblastine/etoposide/ interferon | Progression |
| LR-133 | 15/NA         | LCH | FAM73A-BRAF | Single system, single lesion disease (details not available) | NA | - |
| LR-143 | 14/NA         | Non-LCH | RNF11-BRAF | Brain          | NA | - |
| LR-153 | 38/F          | Non-LCH | CLIP2-BRAF | Retroperitoneum | NA | - |
| LR-163 | 22/F          | AXG | GAS2-BRAF   | Skin, brain (pituitary) | Prednisone | PR |

CR complete response, F female, LR cases from literature review, M male, MC Mayo clinic, NA not available, PR partial response, SD stable disease.
and potentially providing a therapeutic opportunity using MEK inhibitors.

Saurabh Zanwar1, Jithma P. Abeykoon1, Surendra Dasari1, Aishwarya Ravindran2, Jason R. Young3, Aldo A. Acosta-Medina4, Karen L. Rech5, Jonathan Schwartz5, Aaron Mangold6, Allison Rosenthal7, N. Nora Bennani1, Mithun V. Shah1, Diana Moriote8, Gaurav Goyal9✉ and Ronald S. Go1✉

1Division of Hematology, Mayo Clinic, Rochester, MN, USA. 2Division of Hematopathology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA. 3Department of Radiology, Mayo Clinic, Jacksonville, FL, USA. 4Department of Medicine, Mayo Clinic, Rochester, MN, USA. 5Division of Pediatric Hematology/Oncology, Mayo Clinic, Rochester, MN, USA. 6Department of Dermatology, Mayo Clinic, Scottsdale, AZ, USA. 7Division of Hematology, Mayo Clinic, Scottsdale, AZ, USA. 8Division of Hematopathology, University of Alabama at Birmingham, Birmingham, AL, USA. 9Division of Hematology-Oncology, Division of Hematology-Oncology, University of Alabama at Birmingham, Birmingham, AL, USA. ✉email: ggoyal@uabmc.edu; go.ronald@mayo.edu

Fig. 1 Locations of BRAF fusion. Patients in the Mayo Clinic cohort with BRAF fusions were noted to have preserved kinase domain regions.

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AUTHOR CONTRIBUTIONS

SZ, GG, and RSG conceived the study, collected the data, performed the analysis, and wrote the initial draft of the manuscript. AR and KLR generated the figure for breakpoints for BRAF fusions and critically appraised the manuscript. SD generated the pathology image in the supplement and critically reviewed and appraised the manuscript. JPA, JRY, AAA-M, JS, AM, AR, NNB, MVS, and DM critically reviewed and appraised the final draft of the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.