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Molecular Profiling of Tumor Tissue and Plasma Cell-Free DNA from Patients with Non-Langerhans Cell Histiocytosis

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ABSTRACT

The *BRAF*^{V600E} mutation and BRAF inhibitor responsiveness characterize ~50% of patients with the non-Langerhans cell histiocytosis (non-LCH) Erdheim-Chester disease (ECD). We interrogated the non-LCH molecular landscape (ECD, n=35; Rosai-Dorfman disease [RDD], n=3; mixed ECD/RDD, n=1) using *BRAF*^{V600E} polymerase chain reaction and/or next-generation sequencing (tissue and cell-free DNA [cfDNA] of plasma and/or urine). Of 34 evaluable patients, 17 (50%) had the *BRAF*^{V600E} mutation. Of 31 patients evaluable for non-*BRAF*^{V600E} alterations, 18 (58%) had $\geq$1 alteration and 12 putative non-*BRAF*^{V600E} mitogen-activated protein kinase (MAPK) pathway alterations: atypical *BRAF* mutation; *GNAS*, *MAP2K1*, *MAP2K2*, *NF1* and *RAS* mutations*; RAF1* or *ERBB2* amplifications; *LMNA-NTRK1* (TRK inhibitor-sensitive) and *CAPZA2-BRAF f*usions. Four patients had *JAK2, MPL ASXL1*, *U2AF1* alterations, which can correlate with myeloid neoplasms, a known ECD predisposition, and one developed myelofibrosis 13 months after cfDNA testing. Therefore, our multi-modal comprehensive genomics reveals clinically relevant alterations and suggests that MAPK activation is a hallmark of non-LCH.

INTRODUCTION

Non-Langerhans cell histiocytosis (non-LCH) includes rare disorders such as Erdheim-Chester disease (ECD) and Rosai-Dorfman disease (RDD) (1). ECD is a rare CD68-postive, CD1a-negative “L-Group” non-LCH with multi-organ involvement that was initially described in 1930 (1-3). RDD, an “R-group” non-LCH also known as sinus histiocytosis with massive lymphadenopathy, is defined by the accumulation of CD68-postive. S100-positive, CD1a-negative histiocytes in lymph nodes, the dura and less frequently other areas such as skin, bones and soft tissue (1,2). Non-LCH can coexist with LCH (4). Patients with ECD are usually Caucasian men diagnosed in the fifth to seventh decades of life (3,5). The most frequent presentation of ECD includes bone pain due to diffuse sclerotic lesions, which demonstrate foamy lipid-laden histiocytes that predominantly affect the diaphysis of appendicular long bones and often spare the epiphyses. Other common features include orbital

infiltration with proptosis; lung, kidney, retroperitoneal and cardiac involvement, diabetes insipidus, and skin lesions (3,6). RDD is more common in children and young adults of African descent (1). The typical of RDD includes bilateral bulky painless cervical lymphadenopathy associated with fatigue, weight loss, pyrexia and night sweats. Mediastinal, inguinal, and retroperitoneal nodes may also be involved (1,7). Extranodal involvement is present in about 40% of RDD patients and can include the skin, nasal cavity, bone, soft tissue, dura and retro-orbital tissue.

Therapies that have been used successfully for ECD and RDD include interferon-alpha, anakinra (interleukin-1 receptor antagonist), cladribine, and imatinib (8-11). Of interest, about half of the patients with ECD have the *BRAF*^{V600E} (12). Patients with *BRAF*^{V600}-mutated ECD respond to BRAF inhibitors such as dabrafenib or vemurafenib, which was recently approved for ECD by the United States Food and Drug Administration (13,14). In addition, translational studies and anecdotal clinical reports suggested that patients with ECD without *BRAF*^{V600E} mutation and patients with RDD can have other molecular alterations in the mitogen-activated protein kinase (MAPK) pathway such as *KRAS*, *NRAS*, and *MAP2K1* mutations and potentially respond to MEK inhibitors (15-18).

To date, however, the clinical molecular profiling reported on patients with non-LCH has been mainly confined to small gene sets. In addition, molecular testing of tumor tissue has been complicated due to low percentage of tumor cells present in the archival samples, which is a frequent problem in the case of ECD, especially when bone biopsies containing a stroma-rich microenvironment are sampled (19,20). Herein, we describe, the molecular profiling by polymerase chain reaction (PCR) and next generation sequencing (NGS) of tissue and/or blood and/or

urine cell-free DNA (cfDNA) from 39 patients with non-LCH (**Figure 1)**.

MATERIALS AND METHODS

Patients

We identified patients with non-LCH (ECD, RDD), who were treated at MD Anderson Cancer Center (MD Anderson), Memorial Sloan Kettering Cancer Center (MSK) or the University of California San Diego Moores Cancer Center (UCSD) and whose tumor tissue and/or plasma- and/or urine-derived cfDNA was subject to clinical molecular testing. Patients' demographic information was obtained from their electronic medical records. This study was conducted in compliance with the

Declaration of Helsinki and the clinical protocols (MD Anderson LAB10-0334, LAB10-0441 and RCR04-567; UCSD NCT02478931; and MSK 14-201) were approved by the institutional review boards. Written informed consent was obtained from all patients before any study-related procedures were performed.

Molecular profiling

All molecular profiling of tumor tissue and plasma- or urine-derived cfDNA sequencing was performed in Clinical Laboratory Improvement Amendment (CLIA)-certified laboratories.

Tumor tissue: DNA was extracted from microdissected paraffin-embedded tumor sections and analyzed using a PCR-based DNA sequencing method for the *BRAF*^{V600} mutation and/or with the targeted Foundation One or Foundation One Heme NGS assay (Foundation Medicine, Cambridge, MA; http://www.foundationone.com/; hybrid-capture-based comprehensive genomic profiling to a median depth of coverage of >500X testing for 182, 236, 315, or 406 genes, depending on the time and panel) or targeted NGS with the 468 genes MSKCC IMPACT assay (21-23). Alterations captured by targeted NGS included base-pair substitutions, insertions/deletions, copy-number alterations and rearrangements. Alterations likely or known to be *bona fide* oncogenic drivers were included and germline polymorphisms were excluded.

Plasma-derived cfDNA: Circulating cfDNA was extracted from whole blood collected in 10 mL Cell-Free DNA BCT tubes (Streck, Omaha, NE). After double ultracentrifugation, 5 to 30 ng of cfDNA was isolated for digital sequencing (54 to 73 genes) in a CLIA-certified, College of American Pathologists-accredited laboratory using the Guardant360 assay (Guardant Health, Redwood City, CA) as described

previously (24). Both leukocyte- and tumor-derived cfDNA fragments were simultaneously sequenced. The variant allele fraction was calculated as the proportion of cfDNA harboring the variant in the background context of wild-type cfDNA. The analytical sensitivity of the methodology permitted the identification of 1 to 2 mutant fragments in a 10 ml blood sample (0.1% limit of detection) with an analytic specificity > 99.9999%. Gene copy number in plasma is a function of both copy number in tissues and the degree to which tumor DNA was shed into circulation. Gene copy number of 2.5-4.0 are reported as ++ amplification, and those over 4.0 are reported as +++ amplification, representing the 50th-90th and >90th percentiles, respectively of the copy number call in the Guardant360 database (24).

Urine-derived cfDNA: Urine-derived cfDNA was isolated and tested for the presence of the *BRAF*^{V600E} mutation using a droplet digital PCR (ddPCR; QX-100, BioRad; Hercules, CA) assay for cfDNA quantification and rare *BRAF*^{V600E} allele detection (19,20). The RainDrop ddPCR instrument (RainDance; Billerica, MA) was used for PCR droplet separation, fluorescent reading, and counting droplets containing mutant sequences, wild-type sequences, or unreacted probes. For a given patient sample, the assay reported the *BRAF*^{V600E} mutation fragments it detected as a percentage of the wild-type *BRAF*. Thresholds were defined as no detection – wild-type (<0.05%), indeterminate (0.05% - 0.107%), and detected – *BRAF*^{V600E} (>0.107%). All testing was performed in a CLIA-certified laboratory (Trovagene, San Diego, CA).

RESULTS

Patients

We evaluated 39 patients, treated at MD Anderson (n=24), MSK (n=9) or UCSD (n=6). Of these 39 patients, 35 (90%) had ECD, 3 (8%) had RDD and one (2%) had mixed ECD and RDD. Most patients were men (n=22, 56%), Caucasians (n=27, 69%), had disease involving multiple organ systems (n=20, 51%). The median patient age was 49 years (range, 15-76). Patients' characteristics are listed in **Table 1.**

Molecular profiling

Molecular profiling with tumor tissue targeted NGS and/or tumor tissue PCR sequencing and/or plasma-derived cfDNA targeted NGS and/or urine-derived cfDNA PCR was attempted for all 39 patients and yielded at least one valid result (meaning successful test) for the *BRAF*^{V600E} mutation and/or other alterations for 34 (87%) patients (**Figure 1**). The median turnaround times from request to results were 29 days (range, 10-116 days) for tumor tissue targeted NGS, 10 days (5-41 days) for tumor tissue PCR, 13 days (8-18 days) for plasma cfDNA targeted NGS and 16.5 days (7-25 days) for urine cfDNA PCR (P<0.001, **Table 1**).

Tumor tissue targeted NGS: Tumor tissue targeted NGS was attempted for 29 patients and yielded valid results for 22 (76%). The median number of alterations detected excluding variants of unknown significance (VUS) was 1 (range, 0-3 alterations) (**Table 2**). Only 3 (10%) of the 29 patients who were successfully tested had ≥2 alterations (**Figure 2**).

Tumor tissue PCR for the *BRAF*^{V600E} mutation: Of 18 patients tested, 14 (78%) yielded valid results and 10 (56%) showed *BRAF*^{V600E} mutation.

Plasma-derived cfDNA targeted NGS: Testing of plasma samples from all 27 patients yielded a valid result. In addition, 18 (67%) of these 27 patients also had successful tumor tissue targeted NGS testing and 11 (41%) tumor tissue PCR for the *BRAF*^{V600E} mutation (**Table 3**). The median number of alterations detected in plasma-derived cfDNA excluding VUS was 1 (range, 0 – 5 alterations) (**Table 2**). Only 5 (19%) of the 27 patients tested had $\geq$2 alterations (**Figure 2**).

Urine-derived cfDNA for *BRAF V600E*: Samples from all five patients yielded a valid result and one sample had the *BRAF*^{V600E} mutation.

BRAF^{V600E} mutation

Of 39 patients, 34 (87%) had valid molecular testing results for the *BRAF*^{V600E} mutation by any method. Of these 34 patients, 17 (50%) had the *BRAF*^{V600E} mutation. All patients with the *BRAF*^{V600E} mutation had ECD. Tumor tissue testing by either PCR or NGS detected the *BRAF*^{V600E} mutation in all 17 patients. Of interest, 7 patients had valid results for the *BRAF*^{V600E} mutation from both tissue PCR and tissue NGS and while all PCR results revealed *BRAF*^{V600E} mutation, none of the targeted NGS results did (**Table 3**).

Among the 27 patients for whom plasma-derived cfDNA targeted NGS demonstrated valid results, 7 (26%) had the *BRAF*^{V600E} mutation, which was confirmed by tumor tissue PCR or NGS (**Table 3**). Overall, plasma-derived cfDNA and tumor tissue PCR or NGS yielded valid test results for the *BRAF*^{V600E} mutation for 22 patients, resulting in an agreement rate between plasma and tumor tissue of 73% (kappa, 0.49; 95% confidence interval [CI], 0.19-0.79), a sensitivity for plasma of 54%, and a specificity for plasma of 100% (**Table 4**). Of interest, the median time between sample collection for tumor tissue testing (NGS and/or PCR) and plasma-

derived cfDNA testing for 7 patients with the *BRAF*^{V600E} mutation in both plasma-derived cfDNA and tumor tissue (0 weeks; range, -2 to 164 weeks) was shorter than the median time between sample collection for 6 patients with the *BRAF*^{V600E} mutation in tumor tissue but not plasma-derived cfDNA (21 weeks; range, -2 to 69 weeks, $P = 0.045$). In addition, 3 of the 6 patients with the *BRAF*^{V600E} mutation in tumor tissue but not plasma-derived cfDNA received a BRAF or MEK inhibitor between the times of tissue and plasma collection.

Finally, of the 5 patients whose urine-derived cfDNA was tested for the *BRAF*^{V600E} mutation by PCR, 1 had the *BRAF*^{V600E} mutation; this patient also had the *BRAF*^{V600E} mutation in tumor tissue (detected by NGS) and in plasma-derived cfDNA (**Table 3**). Of the 4 remaining patients, who did not have the *BRAF*^{V600E} mutation in urine-derived cfDNA 2 had the *BRAF*^{V600E} mutation in tumor tissue (detected by PCR).

Genomic alterations other than *BRAF*^{V600E} mutation

Of 39 patients, 31 (79%) had valid molecular testing results for alterations other than the *BRAF*^{V600E} mutation by any method and 18 (58%) had 1 or more such alterations (**Tables 2 and 3**). Interestingly, we observed atypical alterations affecting *BRAF*, including a *CAPZA2-BRAF* fusion in an RDD patient and atypical activating *BRAF*^{L485W} mutation in an ECD with the *BRAF*^{V600E} mutation. We also detected fusions such as *MIR143HG-NOTCH2* and *LMNA-NTRK1*. Of 18 patients with molecular alterations other than (n=7) or in addition to the *BRAF*^{V600E} mutation (n=5), 12 (67%) had one or more alterations that putatively directly or indirectly activate the MAPK pathway including *KRAS* mutations (n=2), *NRAS* mutations (n=2), *NF1* mutations (n=3), a *BRAF* atypical mutation or fusion (n=2), a *GNAS* mutation (n=1), an *ERBB2* amplification (n=1), an *RAF1* amplification (n=1), an *LMNA-NTRK1*

fusion (n=1), an *MA*P2K1 mutation (n=1) and an *MAP2K2* mutation (n=1). In addition, we detected several other unique alterations, such as an *MITF* amplification (previously described in melanoma); a *SOX2* amplification (associated with squamous tumors); *JAK2*^{V617F} and *MPL*^{W515L} mutations (usually seen in myelofibrosis*); ASXL1* mutations, which can occur in clonal hematopoiesis of indeterminate potential; and a *U2AF1* mutation (associated with myelodysplastic syndrome) (25-29).

Overall, of 18 patients with valid tumor tissue and plasma-derived cfDNA test results for alterations other than the *BRAF*^{V600E} mutation, 7 (39%) had plasma-derived cfDNA and tumor tissue NGS results that were in complete agreement (**Table 3**). Of interest, the median time between sample collection for tumor tissue and plasma-derived cfDNA testing for these 7 patients was 0 weeks (range, -2 [ctDNA collected before the tissue biopsy] to 69 weeks), whereas the median time between sample collection for tumor tissue and plasma-derived cfDNA testing for 11 patients whose tumor tissue and plasma-derived cfDNA NGS results for alterations other than the *BRAF*^{V600E} mutation were in disagreement was 17 weeks (range, -2 to 164 weeks, $P = 0.08$).

Of interest, 3 patients had RDD, and 1 had mixed ECD and RDD. The patient with mixed ECD and RDD had an osteosclerotic tibial lesion typical of ECD, and biopsy of two disparate bone lesions demonstrates areas of RDD-like histopathology (large histiocyte with pale cytoplasm, strong S100 positivity, emperipolesis) and areas of ECD histopathology (scant S100 positivity in abundant foamy histiocytes). This patient had a *MIR143HG-NOTCH2* fusion as the sole alteration. One RDD patient harbored a *CAPZA2-BRAF* fusion (detected by tumor tissue NGS*)* and a *RAF1*

amplification (detected in plasma cfDNA); the other 2 RDD patients had a *GNAS*^{R201C} and *APC*^{E1157fs} mutation, respectively (detected in plasma-derived cfDNA).

DISCUSSION

We interrogated the molecular profiles of 39 patients with non-LCH using tissue and/or plasma- and/or urine-derived cfDNA with NGS and/or PCR-based sequencing technologies. In 5 patients, all testing that was attempted failed owing to inadequate specimens. The median number of characterized alterations per patient was 1 (range, 0 – 3 alterations) for tissue NGS and 1 (range, 0 – 5 alterations) for plasma-derived cfDNA NGS. Among the 34 patients with at least 1 valid test result (including 30 patients with ECD, 3 patients with RDD, and 1 patient with mixed ECD/RDD), 22 distinct genes were found to harbor characterized somatic alterations (VUS excluded).

We detected some common molecular themes, specifically the involvement of the MAPK pathway, in our patients. Indeed, 24 of 34 patients (71%) with valid genomic results had alterations in genes that directly or indirectly activate the MAPK pathway, including 12 patients with the *BRAF*^{V600E} mutation only, 5 with the *BRAF*^{V600E} mutation and other MAPK-activating alterations, and 7 with other MAPK-activating alterations without the *BRAF*^{V600E} mutation. Other than the *BRAF*^{V600E} mutation, alterations activating the MAPK pathway included an atypical *BRAF* mutation and *CAPZA2-BRAF* fusion; mutations in genes such as *GNAS, MAP2K1, MAP2K2, NF1, NRAS,* and *KRAS*; amplifications of *RAF1* and *ERBB2*; and an *LMNA-NTRK1* fusion. We detected the *BRAF*^{V600E} mutation in 50% of tested patients with non-LCH, which is consistent with previously reported data (12). Furthermore, our results validate in the clinical setting early experimental data from Diamond et al. (15), which also demonstrated the presence of oncogenic alterations including mutations and fusions in *BRAF* and other genes activating the MAPK pathway such as *NRAS*, *KRAS*, *MAP2K1*, and *ARAF* in tissue samples from patients with non-LCH.

These data collectively support the hypothesis that the activation of the MAPK pathway is a hallmark of non-LCH, which can be explored therapeutically. Indeed, early clinical data reported that the MEK inhibitor cobimetinib, which effectively reduces MAPK pathway activation, can be effective in patients with ECD or RDD (18,30). Furthermore, in addition to *LMNA-NTRK1* fusion we observed the novel fusions *CAPZA2-BRAF*, and *MIR143HG-NOTCH2*, which have not been reported in non-LCH (15). The protein encoded by *CAPZA2* is the alpha subunit of the barbed-end actin binding protein Cap Z. By capping the barbed end of actin filaments, Cap Z regulates the growth of the actin filaments (31). Previously, *BRAF* fusions involving the intact in-frame BRAF kinase domain, including 20 novel *BRAF* fusions, were observed in 55 (0.3%) of 20,573 tumors across 12 distinct tumor types (32). To our knowledge, a *CAPZA2-BRAF* fusion has not been reported. *MIR143-NOTCH2* fusions have been previously described in 52% of glomus tumors, which are neoplasms of perivascular smooth muscle differentiation (33). MIR143 is a microRNA co-expressed with MIR145, which functions as a potential tumor suppressor. Intriguingly, one of the patients in the present study had an *LMNA-NTRK1* fusion; colorectal and other cancers with such fusions can demonstrate profound responses to NTRK inhibitors (15,34). We also detected an atypical *BRAF*^{L485W} mutation, which was present alongside a *BRAF*^{V600E} mutation. Although its functional consequences *BRAF*^{L485W} mutation are not fully understood, early clinical data suggest sensitivity to MAPK targeting with the ERK inhibitor ulixertinib, as evidenced by radiological partial response in a patient with advanced gallbladder cancer (35). We also observed an *ERBB2* amplification in one patient. Although uncommon, abnormalities in *ERBB* family members, as well as other genes usually associated with solid tumors, have been documented in lymphoid malignancies

(36). The *MITF* and *SOX2* amplifications observed in our dataset are known to be associated with melanoma and squamous malignancies, respectively (25,26). These findings indicate that, despite the common theme of MAPK pathway involvement, some of our patients had unique alterations, a phenomenon that has been described across the cancer field and supports for the need to perform genomic testing and individualize of therapy (37)

One of our patients, had a *JAK2*^{V617F} mutation; this alteration is typical of myelofibrosis. Although the patient had only ECD when the *JAK2*^{V617F} mutation in cfDNA was detected, approximately 13 months later, he developed anemia, thrombocytopenia, and splenomegaly, and a bone marrow biopsy showed myelofibrosis. Interestingly, another patient had an *MPL*^{W515L} mutation in plasma-derived cfDNA. The *MPL*^{W515L} mutation also activates the JAK-STAT pathway and is observed in myelofibrosis; 8 months after the molecular test, the patient's blood counts were normal. We also observed other alterations in genes involving clonal hematopoiesis such as *ASXL1* mutations. Finally, a *U2AF1*^{Q157P} mutation (a gene anomaly sometimes found in poor-risk myelodysplastic syndrome) was also detected. Papo et al. (36) reported a 10% incidence of simultaneous myeloid neoplasms in a large data set of 189 patients with ECD. Our study was neither designed nor powered to elucidate the significance of the above-mentioned alterations as possible early molecular signals of myeloid malignancies, and longer follow-up to help answer this question is warranted (38).

For patients, who had both tumor tissue and plasma-derived cfDNA testing results available, plasma-derived cfDNA testing for the *BRAF*^{V600E} mutation had 100% specificity but only 54% sensitivity. Of interest, the median interval between tumor tissue and plasma collection was longer for discordant samples (P=0.045), and half

of the patients with discordant results received BRAF or MEK inhibitors before the plasma collection. Both of these factors were previously reported to have a lower concordance between molecular testing of plasma-derived cfDNA and tumor tissue (39,40). In addition, in our study, for all 6 patients with the *BRAF*^{V600E} mutation in tumor tissue, but not plasma-derived cfDNA, the *BRAF*^{V600E} mutation was detected by PCR and not NGS did. The cfDNA targeted NGS method demonstrated consistency between analytical and clinical performance with sensitivity of 86% and specificity >99% through multiple clinical utility studies including assessment of concordance with the molecular testing of the tumor tissue in advanced cancers; however, there have been no data specifically for non-LCH.(41,42) Finally, complete agreement rate between plasma-derived cfDNA and tumor tissue for alterations other than the *BRAF*^{V600E} mutation was low (39%) and compared with patients for whom tumor tissue and cfDNA test results for non-*BRAF*^{V600E} alterations were concordant, patients for whom these test results were discordant showed a trend towards having longer times between sample collections (P=0.08).

In clinical practice, performing comprehensive genomic profiling on tissue, blood, and urine is not standard practice. However, these tests are extremely useful in both the diagnosis and treatment of non-LCH. We feel that both tissue and cfDNA NGS should be performed on all these patients. For the 50-60% percent of *BRAF*^{V600E}-negative patients, NGS can find a targetable alterations allowing for effective treatment. Furthermore, serial cfDNA NGS can help identify patients at risk of developing myeloid neoplasms.

In summary, the comprehensive molecular profiling of tumor tissue and cfDNA from patients with non-LCH revealed multiple and often novel molecular alterations. Most of these alterations activate the MAPK pathway, which suggest

that therapeutic targeting of this pathway is an effective strategy for further clinical development. In addition, we found other molecular alterations that can provide additional targets for matched therapies (e.g., *ERBB2* and *NTRK1* alterations), which supports the use of comprehensive molecular profiling technologies in the development of personalized approaches to treating non-LCH. Furthermore, we found that comprehensive molecular profiling of plasma-derived cfDNA has a good specificity for detection of the *BRAF*^{V600E} mutation and can be used as an alternative to tumor tissue testing, especially when tumor tissue is in short supply; however, exposure to prior systemic therapy might reduce this sensitivity. In addition, in patients with alterations other than *BRAF*^{V600E} mutations the agreement rate between plasma-derived cfDNA and tissue is relatively low and since it is unclear, which alteration is a true driver, it might be warranted to attempt testing of both sources of genomic material. Five patients had *BRAF*^{V600E} mutations along with other mutations in the MAPK pathway. However, as previously reported *BRAF*^{V600E} mutations and *RAS* mutations are usually mutually exclusive unless one of the mutations is subclonal.(43-46) We suspect that some of the non-*BRAFV600E* MAPK pathway alterations represent either clonal hematopoiesis or minority subclones, however, we were unable to definitively prove this possibility. Finally, comprehensive molecular profiling of plasma-derived cfDNA detects molecular alterations that are known to be associated with myeloid malignancies. Therefore, given the known association between ECD and myeloid malignances, further investigation of using comprehensive molecular profiling of plasma-derived cfDNA for the early diagnosis of simultaneous myeloid neoplasia in non-LCH patients is warranted.

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Table 1: **Characteristics of Patients and Genomic Sequencing (N = 39)**

Median age at diagnosis (range) in years	49 (15-76)
Sex	
Men	22
Women	17
Ethnicity	
Caucasian	27
African American	4
Hispanic	3

Middle Eastern	3
Asian	1
Other	1
Type of non-Langerhans histiocytosis	
ECD	35
RDD	3
ECD/RDD	1
Classification	
Multisystem	20
CNS dominant	6
Bone dominant	5
Cutaneous dominant	2
Orbital-craniofacial dominant	2
Unknown	2
Cardiac	1
Retroperitoneal dominant	1
Molecular testing (No. of patients)	
Successful molecular testing by any method	34
Failures	5
BRAF^{V600E} mutation	17
BRAF^{V600E} wild-type	17
Tissue PCR/Sanger sequencing attempted	18
Tissue PCR/Sanger sequencing failed	4
Tissue PCR/Sanger sequencing succeeded	14
Tissue PCR/Sanger not attempted	21
BRAF^{V600E} mutation by tissue PCR/Sanger sequencing	10
Median turnaround time in days (range) for tissue PCR/Sanger Sequencing	**10 (5-41)**
Tissue NGS attempted	29
Tissue NGS failure	7
Tissue NGS succeeded	22
Tissue NGS not attempted	10
BRAF^{V600E} mutation by tissue NGS	7
Median turnaround time in days (range) for tissue NGS	**29 (10-116)**
Urine PCR attempted	5
Urine PCR failure	0
Urine PCR succeeded	5
Urine PCR not attempted	34
BRAF^{V600E} mutation by urine PCR	1
Median turnaround time in days (range) for urine PCR	**16.5 (7-25)**
Plasma cfDNA attempted	27
Plasma cfDNA failed	0
Plasma cfDNA succeeded	27
Plasma cfDNA not attempted	12
BRAF^{V600E} mutation by plasma cfDNA	7
Median turnaround time (days) for plasma cfDNA	**13 (8-18)**

Abbreviations: CNS, central nervous system; cfDNA, cfDNA; ECD, Erdheim Chester Disease; NGS, next generation sequencing; PCR, polymerase chain reaction; RDD, Rosai-Dorfman disease

Table 2: **Genomic alterations detected by tissue NGS or plasma-derived cfDNA**

Variable	**Tissue (NGS only), 29 patients***	**cfDNA NGS, 27 patients***
Median (range) number of alterations detected	1 (0-3)	1 (0-5)
Number of patients with 0 alterations detected	6	11
Number of patients with 1 alteration detected	13	11
Number of patients with 2 alterations detected	2	3
Number of patients with 3 alterations detected	1	1

Number of patients with ≥4 alterations detected	0	1 (5 alterations)
Number of failures	7	0
Types of alterations (VUS are excluded)		
	Cumulative number of tissue alterations = 30* (includes tissue NGS or PCR)	**Cumulative number of cfDNA alterations = 25***
APC	-	1
ASXL1	3	-
BRAF	18 (17 with *BRAF*^{V600E}; 1 with a *CAPZA2-BRAF* fusion)	8 (*BRAF*^{V600E} in 7 patients; one of the 7 patients had B*RAF*^{V600E} and *BRAF* ^{L485W})
CCNE1	-	1
CD36	1	-
ERBB2	-	1
GNAS	-	1
JAK2	-	1
KRAS	-	2
MAP2K1	1	-
MAP2K2	1	1
MIR143HG-NOTCH2 fusion	1	-
MITF	1	-
MPL	-	1
NF1	-	3
NRAS	1	1
NTRK1 FUSION *(LMNA-NTRK1)*	1	-
RAF1	-	1
RIT1	-	1
SOX2	1	-
TP53	-	2
U2AF1	1	-

**VUSs were excluded*
Abbreviations: NGS, next generations sequencing; PCR, polymerase chain reaction; VUS, variants of unknown significance

Table 3: Genomic Profiles (VUS excluded)*

Case ID/ Diagnosis	Tissue PCR for ***BRAF*^{V600E}**	Tissue NGS**	Plasma cfDNA NGS (Variant allele frequency or level of amplification)	Urine cfDNA PCR for ***BRAF*^{V600E}**	Institution
1/ECD	Not done	*MAP2K1*^{Q56P}	Not done	Wild-type	MDACC

2/ECD	*BRAF*^{V600E}	Failed	Not done	Not done	MDACC
3/ECD	Wild-type	Not done	Not done	Not done	MDACC
4/ECD	Failed	Not done	Not done	Not done	MDACC
5/ECD	Failed	Not done	Not done	Not done	MDACC
6/ECD	Not done	*BRAF*^{V600E}	Not done	Not done	MDACC
7/ECD	Failed	Not done	Not done	Not done	MDACC
8/ECD	Failed	Not done	Not done	Not done	MDACC
9/ECD	Not done	*BRAF*^{V600E}	*BRAF*^{V600E}, 0.3% *KRAS*^{G12R}, 0.3%	*BRAF*^{V600E}	MDACC
10/ECD	Not done	*LMNA- NTRK1* fusion	Not done	Not done	MDACC
11/ECD	Not done	*BRAF*^{V600E}	Not done	Not done	MDACC
12/ECD	Wild-type	Failed	*NF1*^{R1132H}, 0.5%	Not done	MDACC
13/ECD	Not done	Failed	Not done	Not done	MDACC
14/ECD	Wild-type	Failed	None	Not done	MDACC
15/ECD	*BRAF*^{V600E}	Not done	Not done	Wild-type	MDACC
16/ECD	Wild-type	Failed	None	Wild-type	MDACC
17/ECD	Not done	*BRAF*^{V600E} *ASXL1*^{E635fs*15}	*BRAF*^{V600E}, 1.2% *CCNE1*^{P396L}, 0.2%	Not done	MDACC
18/ECD	Not done	*BRAF*^{V600E}	*BRAF*^{V600E}, 1.7%	Not done	MDACC
19/ECD	*BRAF*^{V600E}	*ASXL1*^{G646fs*12}	*BRAF*^{V600E}, 0.6% *BRAF*^{L485W}, 0.3% *ERBB2* amplification, 1+	Not done	MDACC
20/ECD	Not done	Failed	None	Not done	MDACC
21/ECD	Not done	*NRAS*^{Q61R}	*MPL*^{W515L}, 1.8%	Not done	MDACC
22/RDD	Not done	*CAPZA2-BRAF* fusion	*RAF1* amplification, 3+	Not done	MDACC
23/RDD	Not done	Not done	*APC*^{E1157fs}, 0.4%	Not done	MDACC
24/RDD	Not done	Not done	*GNAS*^{R201C}, 0.1%	Not done	MDACC
25/ECD	*BRAF*^{V600E}	Not done	*BRAF*^{V600E}, 0.2% *NF1*^{H1494Y}, 0.1%	Not done	UCSD
26/ECD	*BRAF*^{V600E}	None	None	Not done	UCSD
27/ECD	Not done	Failed	None	Not done	UCSD
28/ECD	*BRAF*^{V600E}	None	None	Not done	UCSD
29/ECD	Not done	*BRAF*^{V600E} *ASXL1*^{R693} *U2AF1*^{Q157P}	*BRAF*^{V600E}, 0.06% *RIT*^{M90V}, 4.0% *JAK2*^{V617F}, 2.9% *KRAS*^{A59T}, 2.8% *NRAS*^{G60R}, 0.3%	Not done	UCSD
30/ECD	Not done	*CD36*^{L360*}	*NF1*^{I679fs}, 0.3%	Not done	UCSD
31/ECD	*BRAF*^{V600E}	None	*TP53*^{R273H}, 0.4%	Not done	MSKCC
32/ECD	Not done	None	None	Not done	MSKCC
33/ECD	Not done	None	None	Not done	MSKCC
34/ECD	Not done	Not done	None	Not done	MSKCC
35/ECD	*BRAF*^{V600E}	None	*TP53*^{H179R}, 3.5%	Wild-type	MSKCC
36/ECD-RDD	Not done	*MIR143HG-NOTCH2* fusion	None	Not done	MSKCC
37/ECD	Not done	*BRAF*^{V600E}	*BRAF*^{V600E}, 0.3%	Not done	MSKCC
38/ECD	*BRAF*^{V600E}	*SOX2* amplification *MITF* amplification	None	Not done	MSKCC
39/ECD	*BRAF*^{V600E}	*MAP2K2*^{Y134H}	*MAP2K2*^{Y134H}, 20.3%	Not done	MSKCC

**VUSs were excluded*
Abbreviations: NGS, next generations sequencing; PCR, polymerase chain reaction; VUS, variants of unknown significance

Table 4. Agreement between testing for *BRAF*^{V600E} mutation in plasma cell-free DNA testing and tumor tissue (NGS or PCR)

Patients with *BRAF*^{V600E} mutation	***BRAF*^{V600E}**	***BRAF* wild-**

testing of plasma cell-free DNA and tumor tissue (n= 22)	**mutation in tumor tissue**	**type in tumor tissue**
BRAF^{V600E} mutation in plasma cell-free DNA	7	0
BRAF wild-type in plasma cell-free DNA	6	9
Agreement between plasma and tissue	73% (16/22, κ 0.49, 95% CI 0.19-0.79)	
Sensitivity for plasma cell-free DNA	54% (95% CI 0.25-0.81)	
Specificity for plasma cell-free DNA	100% (95% CI 0.66-1.00)	

Abbreviations: NGS, next generations sequencing; PCR, polymerase chain reaction

FIGURE LEGENDS

Figure 1. Of 39 patients with Erdheim-Chester disease (ECD, n=35), Rosai-Dorfman disease (RDD, n=3) and mixed ECD/RDD (n=1) the valid results from at least on method of molecular testing were available for 34 patients. The diagram depicts the distribution and overlap of the testing methods used such as tumor tissue targeted next-generation sequencing (NGS), tumor tissue PCR for the *BRAF*^{V600E} mutation, plasma-derived cell-free (cf) DNA targeted NGS and urine-derived cfDNA PCR for the *BRAF*^{V600E} mutation.

Figure 2. Number of the genomic alterations detected by the targeted next-generation sequencing (NGS) of tumor tissue (A.) and plasma-derived cell-free (cf) DNA (C.). Distribution of the genomic alterations detected by the targeted NGS of tumor tissue (B.) and plasma-derived cfDNA (D.).