


Original Article

Emerging Evidence of BRAFV600E in LCH: The Iranian Experience

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Citation Shamsian BS, Momtazmanesh N, Molaei Tavana P, Kazemi Aghdam M, Malek F, Shirvani A, Najmabadi H, Rostami P, Hashemieh M, Jamee M. Emerging Evidence of BRAFV600E in LCH: The Iranian Experience. Iran J Blood Cancer. 2025 Mar 30;17(1): 13-19.



Article info:

Received: 11 Jan 2025

Accepted: 26 Mar 2025

Published: 30 Mar 2025

Keywords:

Langerhans cell histiocytosis
BRAFV600E
Mortality
Treatment

Abstract

Introduction: Langerhans cell histiocytosis (LCH) is an inflammatory neoplasm of myeloid origin. The pathologic CD1a+/CD207+ cells are characterized when mutations in the mitogen-activated protein kinase (MAPK) pathway (particularly in *BRAFV600E*) are activated and involvement of pulmonary, skeletal, pituitary, and cutaneous is seen. we aimed to evaluate a cohort of pediatric LCH patients regarding *BRAFV600E* mutations.

Methods: Three referral centers between 2009-2020 collected definite LCH patients. The patients classified by the detection of *BRAFV600E* mutations by real-time polymerase chain reaction (RT-PCR) assay, and comparison was done in demographic and clinical manifestations, response to therapy, and outcome.

Results: Among 50 LCH patients, 17 (34%) female and 33 (66%) male, somatic mutations in the *BRAFV600E* gene were detected in 30 (60%) patients and wild-type genotype was seen in 20 (40%) patients. There was remarkable higher frequency of mutation in young children (less than 8 years old particularly < 2 years, $p = 0.024$). In this study, 21 patients (42%) had multi-system involvement, with no significant difference between the *BRAFV600E* positive group (14 out of 21, 66.7%) and the *BRAFV600E* negative group (7 out of 21, 33.3%, $p = 0.380$). Among patients with risk organ involvement, the *BRAFV600E* mutation was present in most cases (7 out of 8), and all four patients with central nervous system involvement had this mutation. Patients with the *BRAFV600E* mutation showed a lower response to treatment, while those without the mutation responded significantly better to first-line therapies. Notably, 6 out of 7 patients who died had the *BRAFV600E* mutation.

Conclusions: In LCH patients, *BRAFV600E* mutation may influence the onset age, sort and intensity of clinical symptoms, level of the response to therapy, and prognosis.

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1. INTRODUCTION

Langerhans cell histiocytosis (LCH), classified as an inflammatory myeloid neoplasia, is a rare disorder. The incidence of LCH ranges from 2.6 to 8.9 per million cases yearly in children and 1 to 2 per million cases yearly in adults [1, 2]. The clinical manifestations of LCH are highly variable, ranging from localized, self-healing disease to life-threatening multi-system involvement. Aggressive clinical presentations are most commonly observed in patients under the age of two, often leading to dissemination to vital organs such as the liver, spleen, and hematopoietic system. Neurological and endocrine complications complicate 5% to 20% of affected patients.

The diagnosis of LCH is confirmed through histopathological and immunohistochemical studies, which reveal the accumulation of CD207+CD1a+ cells in the bone, skin, liver, spleen, and hematopoietic system. The first-line treatment for LCH consists of a combination of prednisolone and vinblastine. For patients who are refractory to first-line therapy or have vital organ involvement, alternative agents such as cladribine and cytarabine may be administered. Hematopoietic stem cell transplantation is considered a last resort for those who do not respond to conventional treatments.

The role of the BRAFV600E mutation in the pathogenesis of LCH was first described in 2010 by Badalian-Very et al. [3]. The pathological CD1a+/CD207+ cells are a hallmark of LCH, driven by activating mutations in the MAPK pathway, particularly BRAFV600E. Evidence indicates that somatic mutations, particularly BRAFV600E, indirectly activate the RAS-RAF-MEK signal transduction pathway in more than two-thirds of patients [4], suggesting the potential for novel targeted therapies for LCH (Figure 1) [5].

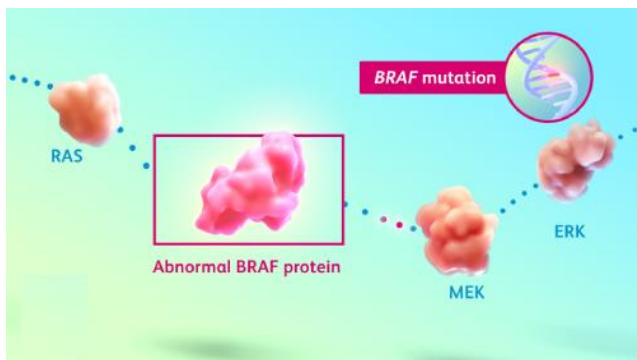


Figure 1. BRAF mutation.

Recently, it has been shown that BRAFV600E mutations are detectable in peripheral blood and can be utilized as a tool for determining disease severity and monitoring

response to treatment [6]. The BRAF V600E mutation is a well-known genetic alteration associated with several types of cancer, especially melanoma, and it can significantly impact treatment strategies. While specific data on BRAFV600E in pediatric ICU patients are limited, understanding the mutation's role in cancer can inform treatment decisions for critically ill children. BRAF mutations, including V600E, are found in various cancers, with notable prevalence in melanoma and colorectal cancer, making them crucial for early diagnosis and targeted therapy in pediatric cases [7].

To our knowledge, no studies have investigated somatic BRAFV600E mutations in Iranian children with LCH. Given the differences in genetic backgrounds among various ethnic groups globally and the potential future use of targeted therapy (such as BRAF inhibitors) in severe LCH, we aim to evaluate a cohort of pediatric LCH patients regarding BRAFV600E mutations.

2. MATERIALS AND METHODS

From 2009 to 2020, a total of 57 pediatric patients with a diagnosis of Langerhans cell histiocytosis (LCH) were identified. Patients were included or excluded based on the availability of tissue samples previously collected for the diagnosis of LCH. Seven patients were excluded due to poor tissue sample quality, resulting in 50 patients enrolled in the study. These patients were treated at Mofid Children's Hospital (n=45), Imam Hossein Hospital (n=3), and Lohman Hakim Hospital (n=2), all affiliated with Shahid Beheshti University of Medical Sciences.

In the genetic laboratory, 50 paraffin blocks of affected tissue underwent DNA extraction to detect the BRAFV600E mutation using a real-time polymerase chain reaction (RT-PCR) assay. Given that normal and affected cells were mixed in the biopsied tissues, a pathologist carefully examined all tissues to select the affected samples for DNA extraction. Estimating the percentage of affected cells is critical for selecting effective genetic testing methods, so the pathologist assessed the proportion of affected tissue in the test samples. A 10-micron section was used for DNA extraction.

To investigate genetic changes at the DNA level, DNA was extracted from the identified affected tissue using the QIAGEN FFPE kit (cat no. 56404). Following quality assurance and confirmation of the samples' appropriate quality, the DNA samples were prepared for PCR. The reverse hybridization strip assay using a BRAF kit (Vienna Lab, REF 5-580) was employed to evaluate the frequency of the BRAFV600E mutation. This assay specifically targeted codon 600 in the BRAF gene.

The reverse hybridization strip assay is a method capable of detecting somatic mutations in this gene through PCR and hybridization reactions. In multiplex PCR, specific biotin-labeled primers were amplified, and the resulting gene fragments were identified in a hybridization reaction with sequence-specific oligonucleotide probes fixed on nitrocellulose strips. Each strip contains a probe for the BRAF mutation and two additional control bands. During hybridization, denatured amplified DNA binds to the gene probes on the strips, and the bands are revealed through a color reaction. The resulting band patterns were analyzed using an evaluation sheet.

A questionnaire was developed to collect clinical information from hospital documents, which included patient demographic characteristics, clinical manifestations, laboratory findings, initial treatment responses, complications, and outcomes. Based on the genetic analysis results, patients were classified into BRAFV600E positive (with somatic mutation) and BRAFV600E negative (wild-type genotype) groups for comparison.

Statistical analyses were conducted using SPSS software (v. 26.0, Chicago, IL). Descriptive statistics included means and standard deviations (SD) for normally distributed data and proportions for categorical variables. Analytical tests, including Mann-Whitney, Chi-square, or Fisher's exact tests, were used for comparisons. A p-value of < 0.05 was considered statistically significant.

3. RESULTS

3.1. Patient Characteristics

The study population included 50 patients, 17 (34%) female and 33 (66%) male, with LCH. Somatic mutations in the BRAFV600E gene were detected in 30 (60%) patients and wild-type genotype was seen in 20 (40%) patients. The mean (\pm SD) age of patients was 4.07 ± 4.5 , 3.83 ± 4.26 years in BRAFV600E positive and 5.12 ± 9.4 in BRAFV600E negative patients.

3.2. BRAFV600E Mutation Frequency

There was remarkable higher frequency of mutation in young children (less than 8 years old particularly < 2 years, $p = 0.024$).

3.3. Organ Involvement

Bone involvement was the most prevalent, occurring in 43 patients (86%), followed by lymph node involvement in 14 patients (28%), skin involvement in 12 patients (24%), and central nervous system involvement in 2 patients (4%). In

50% of the patients (25 patients), the bone was the only organ affected. The degree of bone involvement and the number of affected bones was comparable between the two groups.

Based on the number of organs affected and risk systems involvement (liver, spleen, and bone marrow) patients were classified into four groups as illustrated in **Table 1**. Among eight patients with risk organ involvement, seven had mutations in BRAFV600E.

A total of 8 patients had skull risk bone involvement including temporal, sphenoid, ethmoid, and orbit bones, out of which 6 patients were in the BRAFV600E positive group and only two patients had developed diabetes insipidus (DI). All four patients with central nervous system (CNS) involvement were BRAFV600E positive. Two patients were female and complicated with bone involvement in the hypophysis gland and sella turcica region and manifested DI. Two other patients were male and had intracranial masses, one in the right middle fossa and another in the cellar region, sphenoid sinus, and at the base of the cerebellum.

3.4. Treatment Response

In the current study, 70.2% of patients responded well to first-line treatment with vinblastine and prednisolone, and the rate of overall response was found to be rather lower in BRAFV600E positive patients compared to BRAFV600E negative patients (53% vs. 85%, $p=0.056$). In addition, 11 out of 14 patients who were refractory to first-line treatment were in the BRAFV600E positive group, while 3 out of 14 patients belonged to the BRAFV600E negative group ($p=0.001$). 14 (28%) patients received second-line treatment consisting of cladribine and cytarabine. 7 out of 14 patients (4 in BRAFV600E positive and 3 in BRAFV600E negative group) responded well, while 7 others were resistant to second-line agents (5 in BRAFV600E positive and 2 in BRAFV600E negative group). Seven patients experienced disease relapse (i.e., representing disease manifestations after a period of complete remission during or after discontinuation of treatment). There was no statistically significant difference between BRAFV600E positive ($n=3$) and BRAFV600E negative ($n=4$) groups regarding relapse of the disease ($p=0.39$).

Table 1. Summary of demographic and clinical characteristics among patients with and without BRAFV600E mutation.

Parameters	Total	BRAFV600E Positive (n=30)	BRAFV600E Negative (n=20)	P-value
Sex (M/F), n (%)	33(66.0)/17(34.0)	20(66.7)/10(33.3)	13(65)/7(35)	0.900
Age (years), n (%)				
<2	18 (36.0)	15 (50)	3 (15)	0.024*
2-8	22 (44.0)	9 (30)	13 (65)	
>8	10 (20.0)	6 (20)	4 (20)	
Organ Involvement Category, n (%)				
Single organ w/o pulmonary or RO inv.	29 (58.0)	16 (53.3)	13 (65.0)	0.380
Multiple organs w/o pulmonary or RO inv.	7 (14.0)	4 (13.3)	3 (15.0)	
Multiple organs + \geq one RO inv.	6 (12.0)	3 (10.0)	3 (15.0)	
Single or multiple organs + pulmonary inv.	8 (16.0)	7 (23.3)	1 (5.0)	
Risk Organ Involvement, n (%)				
Liver	4 (8.0)	3 (10.0)	1 (5.0)	0.380
Spleen	3 (6.0)	3 (10.0)	0 (0.0)	
Bone Marrow	1 (2.0)	1 (3.3)	0 (0.0)	
Skull Risk Bone Involvement, n (%)	8 (16.0)	6 (20.0)	2 (10.0)	0.340
CNS Involvement, n (%)	4 (8.0)	4 (13.3)	0 (0.0)	0.140
Response to First-line Treatment, n (%)	33 (70.2)	16 (59.3)	17 (85.0)	0.001*
Relapse, n (%)	7 (14.9)	3 (11.1)	4 (20.0)	0.390
Death Reason, n (%)				
Due to Histiocytosis	6 (12.0)	5 (16.7)	1 (5.0)	0.170
Due to Chemotherapy	1 (2.0)	1 (3.3)	0 (0.0)	

M; male, F; female, Inv: Involvement, RO; Risk Organ, CNS; Central Nervous System

* P-value < 0.05 is considered as significant.

3.5. Survival Data

Overall, seven patients (14%) had ended up with death. The death reason was septic shock (one with chemotherapy-related neutropenia and two others in the context of disease) in three patients, disseminated intravascular coagulation (DIC) (due to LCH-related liver dysfunction) in two, liver encephalopathy in one, and pneumothorax in one patient. Six out of seven deceased patients had mutations in the BRAFV600E gene. According to the Kaplan Meier plot (Figure 2), patients with BRAFV600E mutation had an overall lower survival compared to BRAFV600E negative group, but there was not statistically important difference ($\text{Log rank}=0.189$).

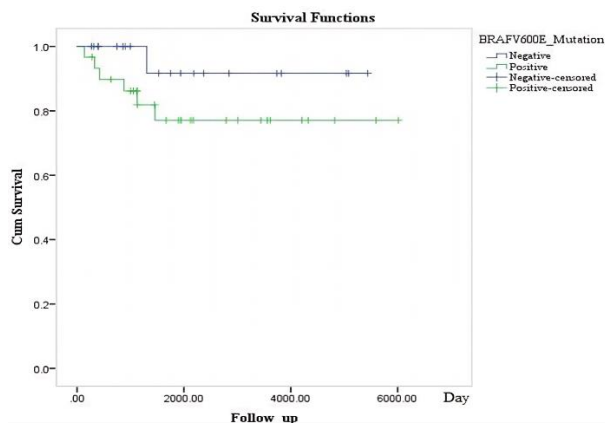


Figure 2. Patients with BRAFV600E mutation had an overall lower survival compared to BRAFV600E negative group ($\text{Log rank}=0.189$).

3.6. Key Findings

Overall, the current study's findings on mutation frequency, organ involvement, treatment responses, disease relapse, and survival enhance the existing understanding of LCH and the implications of BRAFV600E mutations. These results contribute valuable insights into the clinical management and prognostic evaluation of patients with LCH while reinforcing trends observed in previous research.

4. DISCUSSION

In this study, we examined tissue samples for evidence of BRAFV600E somatic mutations in LCH patients diagnosed during the last 10 years and compared patients positive and negative for BRAFV600E somatic mutations in terms of demographic and clinical characteristics and outcomes.

4.1. BRAFV600E Mutation and Age Correlation

The age of patients was reversibly correlated to BRAFV600E mutations and patients less than 8 years old (particularly < 2 years) had higher rates of BRAFV600E mutations ($p=0.024$). Feng et al. found a positive rate of BRAFV600E mutations in 60 of 148 evaluated patients (41%), however there was no correlation between the BRAFV600E gene and gender, onset age, and severity of the disease. However, they introduced onset age equal to less than 2 years old as the

most frequent age range (57%) among their LCH patients [6].

4.2. Organ Involvement

In the current study, 21 (42%) patients suffered from multi-organ involvement, and 14 out of these 21 patients (66.7%) were in the BRAFV600E Positive group. In Bhatia et al. study, the disease in all patients with BRAFV600E mutation was multi-organ or progressive compared to BRAFV600E gene-negative cases (100% versus 41%, $p=0.034$). However, in our study, there was no significant difference for multi-organ involvement between the two groups.

Previous studies have found a positive correlation between risk organ involvement and BRAFV600E mutation [7, 8]. In Héritier et al. study, 87.8% of 315 LCH patients had BRAFV600E mutation with risk organ involvement and represented much poor prognosis compared to other patients. They also identified a significant correlation between skin involvement and BRAFV600E mutation ($P < 0.001$). We found 7 out of 8 patients with risk organ involvement to be positive for BRAFV600E mutation. In addition, 24% of our patients had skin involvement but no significant correlation with BRAFV600E mutation was identified.

4.3. Treatment Response and Prognosis

In the current study, the BRAFV600E positive group showed a generally lower treatment response rate compared to the BRAFV600E negative group. Additionally, patients who tested negative for BRAFV600E mutations were significantly more responsive to first-line agents than those in the BRAFV600E positive group. This is in part consistent with Héritier et al. study reporting a significant correlation between mutated BRAFV600E gene and first-line treatment response ($p=0.001$), with higher response in the BRAFV600E negative compared to BRAFV600E positive group (96.7 vs. 78.1, respectively). The overall response rate to treatment (70.2%) was comparable with the previous study [9] on 400 LCH patients (71%). Nevertheless, only seven patients (14.9%) experienced disease relapse, which was lower than the 27% relapse rate in the Gardner et al. study. Moreover, we did not find any significant difference regarding relapse rate between the two groups, however, Berres et al. in a cohort of 100 LCH patients, reported approximately two folds of relapse rate in BRAFV600E positive in comparison to BRAFV600E negative patients [8]. The limited number of patients in our study may explain this discrepancy and further molecular studies on patients

with LCH are required to be undertaken to achieve a consolidated conclusion. We found a higher mortality rate in patients with BRAFV600E mutation and 6 out of 7 deceased patients had a BRAFV600E mutation. In another study by Zeng et al. on 97 LCH patients, BRAFV600E mutation was introduced as independent prognostic factors of poor disease-free survival (DFS) (hazard ratio [HR]=2.38, 95% confidence interval [CI] 1.02-5.56, $p=0.044$) [10]. Therefore, underlying BRAFV600E mutation may pose further mortality risk on LCH patients. Six of eight (75%) of patients with skull risk bone involvement had BRAFV600E mutation. Although patients with skull risk bone involvement are prone to the development of DI [11], only two of these patients in our study manifested DI and both were in the BRAFV600E positive group.

4.4. CNS Involvement

Aside from central DI, LCH patients may become complicated by a variable spectrum of CNS abnormalities such as neurodegenerative disorders and intracranial tumoral mass [12]. Notably, all four patients with CNS involvement happened to be positive for BRAFV600E mutation. To our knowledge, no study has yet investigated the association between the BRAFV600E mutation positivity and CNS disorders. We hypothesize patients with BRAFV600E mutation are at increased risk of CNS involvement, which requires further confirmation by future studies.

In the current study, we faced several limitations. The study population was reduced from 78 LCH patients to 50 patients due to the unavailability of tissue sample blocks or lack of sufficient tissue cells in the blocks for the purpose of DNA extraction. In addition, the data regarding treatment response was missing in three patients. However, our data represents one of the largest molecular studies from the Iranian population on patients with LCH and can be considered the forefront study that investigated BRAFV600E mutation in this population.

4.5. Study Limitations

The study included only 50 patients, which may limit the generalizability of the findings. A larger sample size could provide more robust statistical power and more comprehensive insights into the variability of clinical outcomes based on BRAFV600E status. The retrospective nature of the study limits the ability to establish causal relationships firmly. Data collection may rely on existing medical records, which can lead to incomplete or

inconsistent information regarding patient symptoms, treatment regimens, and follow-up.

Insufficient longitudinal follow-up may impact the assessment of long-term survival outcomes and the timing and nature of disease relapse. Longer follow-up periods would provide better data on the durability of treatment responses and overall patient prognosis.

5. FUTURE DIRECTIONS

By pursuing these future directions, researchers can further elucidate the implications of BRAFV600E mutations in LCH, which may lead to more effective treatments and improved patient outcomes. A multidisciplinary approach that includes genetics, pathology, treatment modalities, and patient quality of life will be essential in advancing the field.

6. CONCLUSIONS

In conclusion, the clinical significance of BRAFV600E activating somatic mutation in LCH patients is contradictory. BRAFV600E mutation seems to influence the age of onset, type and severity of clinical symptoms, response to therapy and outcome in patients with LCH. Some evidence supports the role of peripheral blood smear BRAFV600E gene evaluation in determining disease severity and response to conventional treatment [13, 14]. Analysis of the BRAFV600E gene at the time of diagnosis and application of BRAFV600E inhibitors may improve treatment response in LCH patients and lead to favorable outcomes.

Ethical statement

The present study was approved by the ethic committee of Research Institute for Children's Health of Shahid Beheshti University of Medical Sciences (Approval Code: IR.SBMU.RICH.REC.1398.011). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgment

We respectfully thank all the patients and their families for their participation in this study. We further extend our gratitude to Mrs. Fahimeh Dini and other staff of the Karimi Nejad-Najmabadi Pathology & Genetics Center for the technical support and

carrying out molecular analysis and Mrs., Zeinab Shormeij for her help in coordinating this project at Pediatric Congenital Hematologic Disorders Research Center in Mofid children hospital.

Funding

This research was partially supported by the Pediatric Congenital Hematologic Disorders Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

- Rodriguez-Galindo C, Allen CE. Langerhans cell histiocytosis. *Blood, The Journal of the American Society of Hematology*. 2020 Apr 16;135(16):1319-31.
- Baumgartner I, von Hochstetter A, Baumert B, Luetolf U, Follath F. Langerhans'-cell histiocytosis in adults. *Medical and Pediatric Oncology: The Official Journal of SIOP—International Society of Pediatric Oncology (Société Internationale d'Oncologie Pédiatrique)*. 1997 Jan;28(1):9-14.
- Badalian-Verly G, Vergilio JA, Degar BA, MacConaill LE, Brandner B, Calicchio ML, Kuo FC, Ligon AH, Stevenson KE, Kehoe SM, Garraway LA. Recurrent BRAF mutations in Langerhans cell histiocytosis. *Blood, The Journal of the American Society of Hematology*. 2010 Sep 16;116(11):1919-23.
- Bigenwald C, Le Berichel J, Wilk CM, Chakraborty R, Chen ST, Tabachnikova A, Mancusi R, Abhyankar H, Casanova-Acebes M, Laface I, Akturk G. BRAF V600E-induced senescence drives Langerhans cell histiocytosis pathophysiology. *Nature medicine*. 2021 May;27(5):851-61.
- Héritier S, Emile JF, Hélias-Rodzewicz Z, Donadieu J. Progress towards molecular-based management of childhood Langerhans cell histiocytosis. *Archives de Pédiatrie*. 2019 Jul 1;26(5):301-7.
- Feng S, Han L, Yue M, Zhong D, Cao J, Guo Y, Sun Y, Zhang H, Cao Z, Cui X, Liu R. Frequency detection of BRAF V600E mutation in a cohort of pediatric langerhans cell histiocytosis patients by next-generation sequencing. *Orphanet Journal of Rare Diseases*. 2021 Jun 11;16(1):272.
- Tang X, Gao J, Guo X, Wan Z, Sun JJ. Beyond BRAFV600E: Investigating the Clinical and Genetic Spectrum of Langerhans Cell Histiocytosis in Children. *Cancer Medicine*. 2024 Dec;13(24): e70532.
- Héritier S, Emile JF, Barkaoui MA, Thomas C, Fraïtag S, Boudjemaa S, Renaud F, Moreau A, Peuchmaur M, Chassagne-Clément C, Dijoud F. BRAF mutation correlates with high-risk Langerhans cell histiocytosis and increased resistance to first-line therapy. *Journal of clinical oncology*. 2016 Sep 1;34(25):3023-30.
- Berres ML, Lim KP, Peters T, Price J, Takizawa H, Salmon H, Idoyaga J, Ruzo A, Lupo PJ, Hicks MJ, Shih A. BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. *Journal of Experimental Medicine*. 2015 Feb 9;212(2):281-.
- Zeng K, Wang Z, Ohshima K, Liu Y, Zhang W, Wang L, Fan L, Li M, Li X, Wang Y, Yu Z. BRAF V600E mutation correlates with suppressive tumor immune microenvironment and reduced disease-

free survival in Langerhans cell histiocytosis. *Oncoimmunology*. 2016 Jul 2;5(7): e1185582.

11. Yeh EA, Greenberg J, Ablá O, Longoni G, Diamond E, Hermiston M, Tran B, Rodriguez-Galindo C, Allen CE, McClain KL, North American Consortium for Histiocytosis. Evaluation and treatment of Langerhans cell histiocytosis patients with central nervous system abnormalities: current views and new vistas. *Pediatric blood & cancer*. 2018 Jan;65(1): e26784.

12. Thacker NH, Ablá O. Pediatric Langerhans cell histiocytosis: state of the science and future directions. *Clin Adv Hematol Oncol*. 2019 Feb 1;17(2):122-31.

13. Schwentner R, Kolenová A, Jug G, Schnöller T, Ahlmann M, Meister B, Lehnbecher T, Minkov M, Hutter C. Longitudinal assessment of peripheral blood BRAF V600E levels in patients with

Langerhans cell histiocytosis. *Pediatric Research*. 2019 May;85(6):856-64.

14. Eckstein OS, Visser J, Rodriguez-Galindo C, Allen CE, NACHO-LIBRE study group. Clinical responses and persistent BRAF V600E+ blood cells in children with LCH treated with MAPK pathway inhibition. *Blood, The Journal of the American Society of Hematology*. 2019 Apr 11;133(15):1691-4.