Supporting Information

Supplementary Methods

Methods for Sequencing

Genomic DNA was extracted from tumor tissue FFPE samples using the truXTRAC FFPE tNA Ultra kit (Covaris; Woburn, Massachusetts, USA) and from a control buccal swab (SW) using QIAamp DNA Mini kit (QIAGEN; Venlo, Limburg, The Netherlands) according to the manufacturers' instructions. The quality of genomic DNA was analyzed using a Qubit Fluorometer (Thermo Fisher) and TapeStation (Agilent). Whole-exome sequencing was performed on a Nextseq 550 platform (Illumina) after enrichment using Magnis Sureselect XT HS Human All Exon V7 probes (Agilent). The average reading level depth for tumor tissue FFPE and SW samples was 86.2× and 117.8×, respectively.

Primary processing of sequencing data was performed using the Genomon 2.6.2 pipeline. Specifically, single nucleotide variants, insertions, and deletions were identified. All data were aligned to the human genome build 19 (hg19). The Integrative Genomics Viewer version 2.11.4 (https://software.broadinstitute.org/software/igv/) was used to visualize and inspect the read alignments and variant calls. Annotations and high-stringency filtering of all variants were completed using an in-house pipeline (Nakamura S, et al. Blood. 2019; 133(25):2682-95). The databases used to annotate variants included RefSeq (http://www.ncbi.nlm.nih.gov/RefSeq/), the 1000 Genomes Project as of August 2015 (http://www.inter natio nalge nome.org/data), dbSNP131 (http://www.ncbi.nlm.nih.gov/projects/SNP/), ToMMo as of August 2020 (https://ijgvd.megabank.tohoku.ac.jp/), the Human Genetic Variation Database as of July 2016 (http://www.hgvd.genom e.med.kyoto -u.ac.jp/), ClinVar as of May 2021 (https ://www.ncbi.nlm. nih.gov/clinv ar/), the Human Gene Mutation Database Professional as of March 2017 (http://www.hgmd.cf.ac.uk/ac/index.php), the cBioPortal for Cancer Genomics as of September 2015 (http://cbioportal.org), the Catalogue Of Somatic Mutations In Cancer version 94 (http://cancer.sanger.ac.uk/cosmic), and the ICGC Data Portal (https://dcc.icgc.org/). The computational algorithms Sorting Intolerant From Tolerant (SIFT,http://sift-dna.org), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/), and MutationTaster (http://www.mutat ionta ster.org/) were used to predict whether mutations were damaging. After adding annotations, single nucleotide mutation and indel data were used to identify LCH-related genes: *BRAF*, *MAP2K*, *KRAS*, *NRAS*, *CSF1R*, *ARAF*, *PIK3CA*, *KIT*, *MAP2K2*, *JAK3*, *PIK3CD*, *RAF1*, *ALK*, *MAPK7*, *MET*, *CSF3R*, and *TEK* (Nat Med. 2019 Dec;25(12):1839-1842.), and we curated pathologically significant mutations based on database registration and functional prediction.

Supplementary Table S1. Additional malignancies

Age at diagnosis years, Cancer		
Preceding LCH diagnosis	Concurrent with LCH diagnosis	After LCH diagnosis
18, Germ cell tumor	40, Thyroid papilla carcinoma	29, Thyroid papilla carcinoma
24, T-LBL	46, Chordoma	45, MDS
30, Gastric cancer	49, Hodgkin lymphoma	61, Oropharyngeal cancer
39, Phyllodes tumor of the breast	60, Adult T cell leukemia	64, LCS
43, Seminoma		67, MDS
47, Cervical cancer		82, AML
54, Spinal cord tumor		
60, Rectal cancer		
75, Cecal cancer		

Abbreviations: T-ALL/LBL, T-lymphoblastic leukemia / lymphoma; MDS, myelodysplastic syndromes; LCS, Langerhans cell sarcoma; AML, acute myeloid leukemia

Supplementary Table S2. Initial treatment in 86 patients with Langerhans cell histiocytosis (LCH) according to disease manifestation pattern

Diagnosis at treatment	Chemotherapy	Immunotherapy[a]	Radiotherapy	Operation	Observation	Other
SSs, n	0	0	3	6	1	0
SSm, n	8	4	1	4	3	2
PLCH, n	0	1	0	1	6[b]	0
MS, n	33	3	1	3	3	3

Abbreviations: SSs-LCH, single system with single-site LCH; SSm-LCH, single system with multiple-site LCH; PLCH, primary pulmonary LCH; MS-LCH, multisystem LCH

[a]Immunotherapy: With prednisolone

[b]Smoking cassation was prescribed

Supplementary Table S3. The lesion site, symptoms at diagnosis, and course of treatment in 13 patients with MS-LCH who did not receive chemotherapy as initial treatment

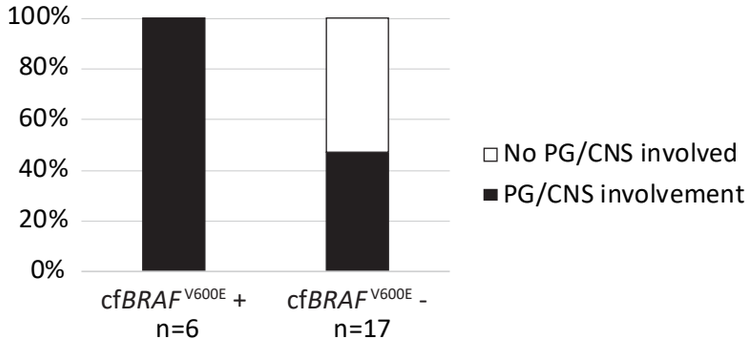
Case	Organ involvement	Symptoms at diagnosis	First line therapy	Second line therapy
1	Bone marrow, Spleen	[a]Without symptom	Observation	-
2	Lung, Liver	[a]Without symptom	Smoking cessation therapy	-
3	Lung, Liver	[a]Without symptom	Smoking cessation therapy	-
4	Skin, PG	Rash	Topical steroid therapy	-
5	Skin, LN	Rash	Topical steroid therapy	-
6	multiple Bones, Skin ,Liver	Rash	Topical steroid therapy	Ultraviolet therapy
7	Bone, Skin	Palpable mass	Systemic steroid therapy	-
8	Bone, Lung, Skin	Cough	Systemic steroid therapy	Chemotherapy
9	Bone, LN, ST	Palpable mass	Systemic steroid therapy	Chemotherapy
10	multiple Bones, PG, CNS, LN	Palpable lymph nodes	Radiation therapy	Chemotherapy
11	Bone, Lung	Bone pain	Operation	Chemotherapy
12	Bone, LN, ST	Bone pain	Operation	Chemotherapy
13	multiple Bones, PG, ST	Bone pain, Palpable mass	Operation	Chemotherapy

Abbreviations: PG, pituitary gland; LN, lymph nodes; ST, soft tissue; CNS, central nervous system

[a]Without symptom: without symptom (abnormal findings on images)

Supplementary Figure S1. Relationship between *BRAF* V600E in plasma cfDNA and PG/CNS involvement in 23 patients.

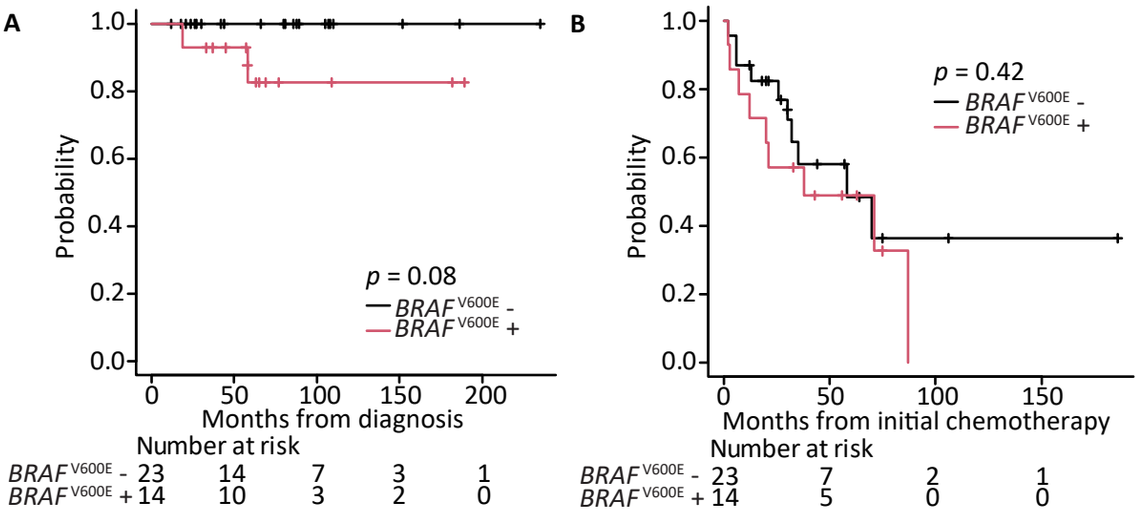
Fisher's exact test, $p = 0.048$



Abbreviations: PG, pituitary gland; CNS, central nervous system, cf*BRAF* ^{V600E}, *BRAF* V600E mutation in plasma cell-free DNA

Supplementary Figure S2. Overall survival (OS) and Event-free survival (EFS) of adult LCH patients with *BRAF* V600E mutation in any of the tests.

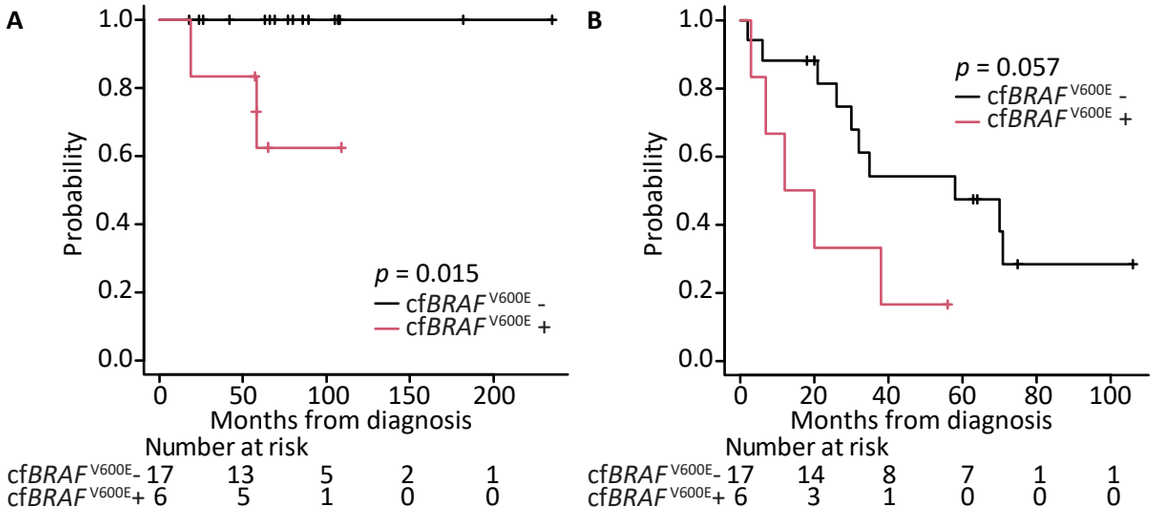
Kaplan–Meier estimation of (A) OS from diagnosis (p = 0.08) and (B) EFS from initial treatment (p = 0.42) of all 37 patients.



Abbreviations: *BRAF* ^{V600E} +, for example, positive for *BRAF* V600E in plasma cell free DNA, positive for *BRAF* V600E in lesion tissues detected using immunohistochemistry, polymerase chain reaction, or whole-exome sequencing.

Figure S3. Overall survival (OS) and Event-free survival (EFS) of adult LCH patients with *BRAF* V600E mutation in plasma cfDNA.

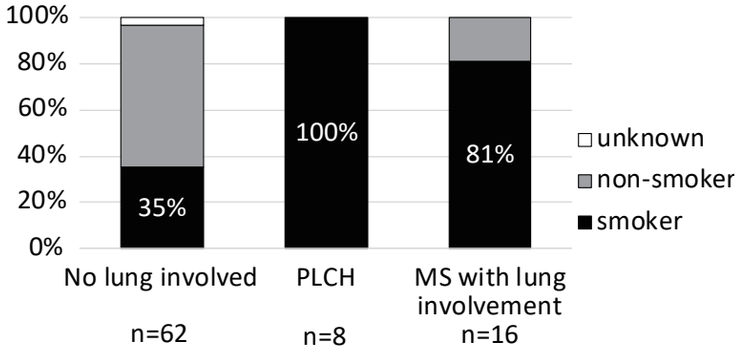
Kaplan–Meier estimation of (A) OS from diagnosis (p = 0.015) and (B) EFS from initial treatment (p = 0.057) of all 23 patients.



Abbreviations: cf*BRAF* V600E, *BRAF* V600E mutation in plasma cell free DNA

Supplementary Figure S4. Relationship between smoking history and lung disease in 86 adult patients with Langerhans cell histiocytosis (LCH).

Fisher's exact test, $p < 0.0001$



Abbreviations: PLCH, primary pulmonary LCH; MS-LCH, multisystem LCH

Supplementary document S1. Questionnaire

This has been provided by the authors to provide readers additional information about their work.

Questionnaire for the Adult LCH patient (Department of Hematology)	
Date	dd/mm/yyyy
Name of Institution	
Person in charge	Dr.
Contact details	e-mail： Tel： Fax：
Characteristics of the Patient	
Patient Registration No	
Date of Birth	dd/mm/yyyy
Sex	1. Male 2. Female
Past Medical History	1. Malignancy：☐Yes, ☐No
	1) (Date: mm/yyyy) Therapy: ☐Operation, ☐Radiation, ☐Chemotherapy, ☐Other (In particular)
	2) (Date: mm/yyyy) Therapy: ☐Operation, ☐Radiation, ☐Chemotherapy, ☐Other (In particular)
	2. Disease except malignancy：☐Yes, ☐No
	1) (Date: mm/yyyy)
	2) (Date: mm/yyyy)
	3) (Date: mm/yyyy)
Social History	Smoking：☐No, ☐Yes (Brinkman Index:)
Family History (only Malignancy)	
Onset / Diagnosis	
Date of Initial Symptom	mm/yyyy
Initial Manifestation	☐Fever ☐Headache ☐Bone Pain ☐Rash ☐Palpable mass ☐Palpable Lymph Node ☐Polyuria / Polydipsia ☐Respiratory Symptoms (eg, Cough, Breathlessness) ☐Visual Impairment ☐Memory Impairment ☐Other( )
Reason for consultation (Except for Initial Symptom)	
Date of First Admission	mm/yyyy
Department on First Admission	☐Internal Medicine(Hematology・Endocrinology・Respiratory Medicine・Gastroenterology・Neurology・Other:) ☐Dermatology ☐Ophthalmology ☐Otorhinolaryngology ☐Oral Surgery ☐Gynecology ☐General Surgery ☐Neurosurgery ☐Orthopedics ☐Other( )
Organ Involvement (Based on imaging system)	☐Skin☐Mucous membrane☐Bone（Sites:) ☐Thymus☐Lung☐Thyroid Gland ☐Bone marrow☐Lymph Node(Sites:) ☐Liver☐Spleen☐Soft tissue ☐Central Nervous System（Except for Pituitary Gland）☐Pituitary Gland ☐Other( )
Place of Biopsy	
Date of Determined Diagnosis	mm/yyyy
Department of Determined Diagnosis	

Histopathological Positivity	☐Only Hematoxylin-Eosin Stain ☐CD1a ☐S100 ☐Langerin ☐Birbeck granules
Genetic Mutations	☐*BRAF*-V600E Mutation: positive, Negative, Unknown (☐Immunohistochemistry ☐Sequence ☐Cell Free DNA) ☐Other(MAP2K1mutation)
Date of First Visit For Your Department	mm/yyyy
Blood Test (Pre-therapy) dd/mm/yy	Blood count: RBC(x10^4/mL), Hb(g/dL), Ht(%), WBC(/mL), PLT(x10^4/mL), White blood cell differentiation: Neu %、Ly %、Mo %、Eo %、Ba %、Other % Biochemical test: CRP(mg/dL), Ig G/A/M(/ / mg /dL), sIL2R( ), AST(IU/L), ALT(IU/L), ALP(IU/L), LDH(IU/L), Alb(g/dL), BAP(Bone Specific Alkaline Phosphatase)(U/L), NTX(Type I collagen cross-linked N-telopeptide( ), TRACP5b(Tartrate-resistant acid phosphatase)( )
Image Test （Pre-therapy）	FDG-PET: ☐Not Administered, ☐Administered (Finding Report:) CT: ☐Not Administered, ☐Administered (Finding Report:) Brain MRI: ☐Not Administered, ☐Administered (Finding Report:) Bone Scintigraphy: ☐Not Administered, ☐Administered (Finding Report:) Gallium Scintigraphy: ☐Not Administered, ☐Administered (Finding Report:)
Physiological Examination	Bone Marrow aspiration/biopsy: ☐Not Administered, ☐Administered (Finding Report:) Gastrointestinal Endoscopy: ☐Not Administered, ☐Administered (Finding Report:)
Treatment/Prognosis	
Treatment	☐Observation the Progress (Include of Smoking Cessation)
1. Symptomatic Treatment	☐Analgesic Agent ☐Antihistaminic Agent ☐Hormone Replacement Therapy：☐ADH, ☐Cortisol, ☐Other( ) ☐Other( )
2. Local Treatment	☐Surgical Resection(Location:) (Date: mm/yyyy) ☐Radiotherapy(Location: ,Total Gy) (Date: mm/yyyy) ☐External Medicine (Steroid) (Location:)
3. Systemic Treatment	☐Steroids Monotherapy (Dose per Day , Dosing Period) ☐Chemotherapy: Duration of Therapy (mm/yyyy- mm/yyyy) ☐Special-C regimen (VBL/PSL+MTX+6-MP) : Total courses ☐Regimen contained with Ara-C (Name of the Drug:) : Total courses ☐Regimen contained with 2-CdA (Name of the Drug:) : Total courses ☐Other (Name of the Drug:) : Total courses ☐Hematopoietic Stem Cell Plantation：Autologous・Allo(Donor type, Conditioning Regimen):
Response at the end of the therapy (For only local therapy or systemic therapy)	☐Non active disease (NAD) Relapse: ☐No,☐Yes Date of Relapse: mm/yyyy- mm/yyyy Relapse sites: Therapy for Relapse: ☐Partial Response (PR) ☐No Response (NR) ☐Progression Disease (PD)
Prognosis at the time of evaluation	☐Alive, ☐Dead (Date of Death: mm/yyyy, Cause of Death: ☐LCH, ☐Other( )

Other	
Questionnaire for the Adult LCH patient (Department of Pathology)	
Date	dd/mm/yyyy
Name of Institution	
Person in charge	Dr.
Contact details	e-mail： Tel： Fax：
Characteristics of the Patient	
Patient Registration No	
Date of Birth	dd/mm/yyyy
Sex	1. Male 2. Female
Onset / Diagnosis	
Date of Initial Symptom	mm/yyyy
Initial Manifestation	☐Fever ☐Headache ☐Bone Pain ☐Rash ☐Palpable mass ☐Palpable Lymph Node ☐Polyuria / Polydipsia ☐Respiratory Symptoms (eg, Cough, Breathlessness) ☐Visual Impairment ☐Memory Impairment ☐Other( )
Reason for consultation (Except for Initial Symptom)	
Date of First Admission	mm/yyyy
Department on First Admission	☐Internal Medicine(Hematology・Endocrinology・Respiratory Medicine・Gastroenterology・Neurology・Other:) ☐Dermatology ☐Ophthalmology ☐Otorhinolaryngology ☐Oral Surgery ☐Gynecology ☐General Surgery ☐Neurosurgery ☐Orthopedics ☐Other( )
Organ Involvement (Based on imaging system)	☐Skin☐Mucous membrane☐Bone（Sites:) ☐Thymus☐Lung☐Thyroid Gland ☐Bone marrow☐Lymph Node(Sites:) ☐Liver☐Spleen☐Soft tissue ☐Central Nervous System（Except for Pituitary Gland）☐Pituitary Gland ☐Other( )
Place of Biopsy	
Date of Determined Diagnosis	mm/yyyy
Histopathological Positivity	☐Only Hematoxylin-Eosin Stain ☐CD1a ☐S100 ☐Langerin ☐Birbeck granules
Genetic Mutations	☐*BRAF*-V600E Mutation: positive, Negative, Unknown (☐Immunohistochemistry ☐Sequence ☐Cell Free DNA) ☐Other(MAP2K1mutation)