SYSTEMATIC SEARCH AND META-ANALYSIS

Methods

A search in 5 databases (Embase.com, Medline ALL Ovid, Web of Science Core Collection, Cochrane Central register of Trials and Google Scholar) up to June 2022 was performed with the search terms described below. All English articles following the search result were screened on title and abstract by two independent authors (TCEZ and PGK). The full text of all selected articles was then assessed and data extracted when fulfilling the following inclusion criteria: A) LCH diagnosis was based on standard histological evaluation; B) age of 18 years or older at LCH diagnosis. For studies on a mixed cohort of adults and children, only data on adult patients was extracted; C) tissue genotyping was performed by PCR- or sequencing-based techniques. Cases with mutational analysis exclusively based on BRAF-VE1 immunohistochemistry (IHC) were not included due to its reported variable specificity and sensitivity[1]; and D) the number of reported adult patients in the study was three or more. Conference abstracts were excluded from the study due to the lack of peer review. Where applicable, an extra reference check was done to search for articles that were missed by our search strategy. Data were extracted by one author (TCEZ) and verified by another (PGK) including age of study participants, *BRAF*^{V600E} testing technique, prevalence of detected mutations, disease extent (single system (SS) pulmonary, SS non-pulmonary and multisystem disease) and outcome. Outcomes were categorized into 'event' and 'non-event' groups, with an event being defined as disease progression, relapse, or death by any cause.[2]

Data obtained by the literature search were combined with data from our own adult LCH cohort. Given the reported validation of routine BRAF-VE1 immunostaining in the pathologic analysis of histiocytic neoplasms within one of the participating institutions[1], patients molecularly analyzed exclusively by BRAF-VE1 IHC in this institution were included in the meta-analysis. Dutch cases were all analyzed by PCR or DNA sequencing. Confidence intervals for mutational frequencies were calculated with normal approximation (Wald) statistics. Additionally, from the combined dataset forest plots were constructed by using the 'Meta-Essentials' tool (workbook "*Differences between independent groups – binary data*") with the random effects model.[3] The Mantel-Haenszel weighting method was applied. An odds ratio (OR) was calculated for which the confidence interval is set at 95%. A result was considered significant following a two-tailed *P* value <0.05. In addition, heterogeneity was assessed by the I^2 statistic.[4]

Results

The literature search generated a total of 2067 published reports. Of these, 171 were selected based on the title and abstract. Upon further review of the full text, 24 articles were eligible for our final analysis, all of which presented data on frequencies of one or more pathogenic variants. A *BRAF*^{V600E} mutation was found in 277/712 of patients (38.9%, 95% CI 35.3%-42.5%), while a *MAP2K1* mutation was reported in 35/267 (13.1%, 95% CI 9.1% - 17.2%) of *BRAF*^{V600E} negative patients. Twelve reports provided sufficient information to be included in a meta-analysis addressing associations of *BRAF*^{V600E} with MS disease and five reports were included in a meta-analysis addressing associations of *BRAF*^{V600E} with events (Figure S6; Table S7). *BRAF*^{V600E} did not correlate with MS disease (OR 0.75, 95% CI 0.53 – 1.05, *P* = .07, Figure S7A). When excluding SS pulmonary LCH from the analysis, this lack of correlation remained unaffected (OR 0.71, 95% CI 0.44 – 1.16, *P* = .12, Figure S7B). Likewise, the meta-analysis could not demonstrate an association between *BRAF*^{V600E} and occurrence of any event (OR 0.80, 95% CI 0.54 – 1.18, *P* = .14, Figure S7C). For all forest plots there was no observed heterogeneity, demonstrated by an I^2 = 0%.

Search terms

Embase.com (1971-)

('Langerhans cell histiocytosis'/de OR ('Langerhans cell'/de AND 'histiocytosis'/de) OR ((Langerhans* NEAR/6 (histiocytos* OR granulomatos*) NOT non-langerhans) OR (letterer NEAR/6 (siwe OR sive OR christian OR reticulose)) OR histiocytosis-X):ab,ti) AND ('genetics'/exp OR 'familial disease'/de OR 'inheritance'/exp OR 'heritability'/exp OR 'mutation'/exp OR 'B Raf kinase'/de OR 'Raf protein'/de OR 'b raf gene'/de OR 'microtubule associated protein 2'/de OR 'microtubule associated protein 3'/de OR 'araf gene'/de OR 'gene'/exp OR 'twins'/exp OR 'twin study'/de OR 'molecular genetic phenomena and functions'/exp OR 'clonal variation'/de OR 'genetic

procedures'/exp OR 'genetic parameters'/exp OR 'DNA'/exp OR (genetic* OR familial* OR inheritan* OR heritab* OR mutation* OR braf OR raf OR map2k1 OR map2-k1 OR map-2k1 OR map-2-k1 OR map3k1 OR map3-k1 OR map-3k1 OR map-3-k1 OR araf OR gene OR genes OR twin* OR genomic* OR telomere* OR translocation* OR clonality OR clonal OR genome* OR gwas OR oncogene* OR V600E OR V600-E OR V-600E OR V-600-E OR MEK OR MEK1 OR MEKK1 OR mutant* OR deletion* OR epigentic* OR dna OR desoxyribonucle*):Ab,ti) NOT (child/exp NOT (adult/exp OR adolescent/exp)) AND [english]/lim NOT ([animals]/lim NOT [humans]/lim)

Medline ALL Ovid (1946-)
(Histiocytosis / OR (Langerhans Cells / AND Histiocytosis /) OR ((Langerhans* ADJ6 (histiocytos* OR granulomatos*) NOT non-langerhans) OR (letterer ADJ6 (siwe OR sive OR christian OR reticulose)) OR histiocytosis-X).ab,ti.) AND (exp Genetics/ OR Genetics.fs. OR Genetic Diseases, Inborn / OR exp Inheritance Patterns / OR exp Mutation / OR exp raf Kinases / OR exp Microtubule-Associated Proteins/ OR exp Genes / OR exp Twins / OR Twin Study/ OR Twin Studies as Topic / OR exp DNA/ OR (genetic* OR familial* OR inheritan* OR heritab* OR mutation* OR braf OR raf OR map2k1 OR map2-k1 OR map-2k1 OR map-2-k1 OR map3k1 OR map3-k1 OR map-3k1 OR map-3-k1 OR araf OR gene OR genes OR twin* OR genomic* OR telomere* OR translocation* OR clonality OR clonal OR genome* OR gwas OR oncogene* OR V600E OR V600-E OR V-600E OR V-600-E OR MEK OR MEK1 OR MEKK1 OR mutant* OR deletion* OR epigentic* OR dna OR desoxyribonucle*).ab,ti.) NOT ((exp child/ OR exp infant/) NOT (exp adult/ OR adolescent/)) AND english.la. NOT (exp animals/ NOT humans/)

Web of Science Core Collection (1971-)
TS=((((Langerhans* NEAR/5 (histiocytos* OR granulomatos*) NOT non-langerhans) OR (letterer NEAR/5 (siwe OR sive OR christian OR reticulose)) OR histiocytosis-X)) AND ((genetic* OR familial* OR inheritan* OR heritab* OR mutation* OR braf OR raf OR map2k1 OR map2-k1 OR map-2k1 OR map-2-k1 OR map3k1 OR map3-k1 OR map-3k1 OR map-3-k1 OR araf OR gene OR genes OR twin* OR genomic* OR telomere* OR translocation* OR clonality OR clonal OR genome* OR gwas OR oncogene* OR V600E OR V600-E OR V-600E OR V-600-E OR MEK OR MEK1 OR MEKK1 OR mutant* OR deletion* OR epigentic* OR dna OR desoxyribonucle*)) AND (adult*)) AND LA=(english)

Cochrane CENTRAL register of Trials (1992-)
(((Langerhans* NEAR/6 (histiocytos* OR granulomatos*) NOT non NEXT langerhans) OR (letterer NEAR/6 (siwe OR sive OR christian OR reticulose)) OR histiocytosis NEXT X):ab,ti) AND ((genetic* OR familial* OR inheritan* OR heritab* OR mutation* OR braf OR raf OR map2k1 OR map2 NEXT k1 OR map NEXT 2k1 OR map NEXT 2 NEXT k1 OR map3k1 OR map3 NEXT k1 OR map NEXT 3k1 OR map NEXT 3 NEXT k1 OR araf OR gene OR genes OR twin* OR genomic* OR telomere* OR translocation* OR clonality OR clonal OR genome* OR gwas OR oncogene* OR V600E OR V600 NEXT E OR V NEXT 600E OR V NEXT 600 NEXT E OR MEK OR MEK1 OR MEKK1 OR mutant* OR deletion* OR epigentic* OR dna OR desoxyribonucle*):Ab,ti) AND (adult*)

Google scholar
"Langerhans* histiocytosis"
genetics|familial|inheritance|heritability|mutation|gene|genes|twins|genomics|telomere|translocation|clonality|clonal|genome|gwas|oncogene adult|adults -"non Langerhans"

SUPPLEMENTARY TABLE 1. Clinical characteristics according to subcohort

	Dutch cohort	Mayo Clinic cohort	*P* value
Patients	71	85	
Age at diagnosis, median (range)	36.3 years (18 – 73)	42.2 years (19 – 88)	0.07
Sex			
Male	40 (56.3%)	46 (54.1%)	0.87
Female	31 (43.7%)	39 (45.9%)	
***BRAF*^{V600E} positive**	34 (47.9%)	40 (47.1%)	1
Disease extent at diagnosis			
Multisystem	50 (70.4%)	48 (56.5%)	0.10
Single system	21 (29.6%)	37 (43.5%)	
Detailed subtype[$]			
MS, RO+[£]	3 (4.2%)	12 (14.1%)	0.05
MS, RO-	18 (25.4%)	25 (29.4%)	0.59
SS, bone	34 (47.9%)	24 (28.2%)	**0.013**
• SS, UFB	30 (42.3%)	18 (21.2%)	**0.005**
• SS, MFB	4 (5.6%)	6 (7.1%)	0.76
SS, skin	2 (2.8%)	9 (10.6%)	0.07
SS, lung	11 (15.5%)	11 (12.9%)	0.65
SS, other	3 (4.2%)	4 (4.7%)	1
Disease site(s) at diagnosis			
Bone	50 (70.4%)	52 (61.2%)	0.24
Lung	26 (36.6%)	30 (35.3%)	0.87
Skin	4 (5.6%)	21 (24.7%)	**0.002**
Central nervous system[#]	5 (7.0%)	13 (15.3%)	0.13
Lymph node	8 (11.3%)	10 (11.8%)	1
Risk organ[£]	3 (4.2%)	13 (15.3%)	**0.032**
Gastrointestinal tract	1 (1.4%)	2 (2.4%)	1
First-line therapy			
Chemotherapy*	10 (14.1%)	32 (37.6%)	**0.001**
Radiotherapy	3 (4.2%)	12 (14.1%)	0.05
Targeted therapy	0 (0.0%)	4 (4.7%)	0.13
None / other therapy	58 (81.7%)	36 (42.4%)	**<0.001**
Unknown	0 (0.0%)	1 (1.2%)	1
Follow-up, median (range)	7.4 years (0 – 36)	2.9 years (0 – 25)	**<0.001**

Symbols: [$] Fisher's exact tests comparing patients with vs. without a disease extent subtype are shown. [£] The hematopoietic system, liver, and spleen were considered risk organs – extrapolating from pediatric data. [#] Given that the posterior pituitary and pituitary stalk are direct extensions of the hypothalamus, pituitary tumors are classified as CNS involvement. * With or without additional local therapy. Abbreviations: SS, single system; UFB, unifocal bone; MFB, multifocal bone; MS, multisystem; RO, risk organ; N/A, not available.

SUPPLEMENTARY TABLE 2. Clinical characteristics of patients with MAPK gene alterations other than *BRAF*^{V600E}

Nr	MAPK pathway gene alteration	Sex	Age[#] (years)	Disease extent	Site(s) of disease at diagnosis	First-line chemotherapy or targeted therapy	Progression/relapse (Time to first event)	Additional malignancy (Time of diagnosis)	Follow-up (years)
	***MAP2K1* (NM_002755.3)**								
NL11	p.E102_I103del	F	51	MS-RO-	Bone, pituitary	Yes: VBL/Pred/6-MP	Yes (2.8 years)	No	15.2
NL24	p.E102_I103del	M	51	MS-RO-	Bone, lungs	No	Yes (4.3 years)	Yes: IMT (5.0 years before LCH)	14.3
NL52	p.E102_I103del	M	58	MS-RO-	Bone, lymph nodes	Yes: VBL/Pred/6-MP	Yes (3.7 years)	No	8.2
NL69	p.E102_I103del	F	62	MS-RO-	Skin, lungs	No	No	No	0.8
NL68	p.E102_I103del	M	28	SS-CNS	CNS	No	No	No	0.7
NL17	p.E102_I103del	M	45	SS-MFB	Bone	No	Yes (0.9 years)	No	22.4
NL66	p.P105_A106del	F	47	SS-MFB	Bone	No	Yes (1.4 years)	No	1.7
US190	p.E102_I103del	M	49	SS-MFB	Bone	No	No	No	2.6
NL55	p.Q56_G61delinsR	M	18	SS-UFB	Bone	No	No	No	2.3
NL36	p.K57_G61del	M	22	SS-UFB	Bone	No	No	No	1.1
NL38	p.E102_I103del	F	22	SS-UFB	Bone	No	Yes (3.6 years)	No	4.3
NL37	p.L101_I103delinsF	M	24	SS-UFB	Bone	No	No	No	3.4
NL1	p.E102_I103del	M	26	SS-UFB	Bone	No	Yes (1.2 years)	No	8.2
NL35	p.Q56_G61delinsR	M	27	SS-UFB	Bone	No	No	No	5.1
US206	p.Q56P	M	27	SS-UFB	Bone	No	No	No	1.0
NL50	p.E102_I103del	F	27	SS-UFB	Bone	Yes: cytarabine[$]	No	No	3.0
NL21	p.E102_I103del	F	30	SS-UFB	Bone	No	Yes (0.5 years)	No	15.3
NL7	p.H100_I103delinsPL	F	38	SS-UFB	Bone	No	No	No	11.9
NL13	p.Q56P	F	42	SS-UFB	Bone	No	No	No	9.3
NL54	p.E102_I103del	M	64	SS-lung	Lungs	No	No	No	0.2
	***BRAF* (NM_004333.6)**								
US216	p.N486_P490del	M	64	MS-RO-	Bone, skin, lungs, pituitary	Yes: vemurafenib	No	Yes: PTC (0.1 years after LCH)	0.3
US219	p.N486_P490del	F	40	SS-skin	Skin	No	No	Yes: melanoma (5.1 years before LCH)	0.2
US114	p.R662K	F	55	SS-UFB	Bone	Yes: cladribine[$]	No	No	9.8
US208	*LMTK2::BRAF*	F	54	SS-MFB	Bone	No	Yes (0.3 years)	No	1.0

All of these 24 patients were tested negative for *BRAF*^{V600E}. All patients were alive at last follow-up. Symbols: [#] Age at diagnosis. [$] Both of these patients with SS-UFB LCH had CNS-risk bone lesions. Abbreviations: IMT, inflammatory myofibroblastic tumor; PTC, papillary thyroid carcinoma.

SUPPLEMENTARY TABLE 3. Multivariable Cox regression analysis of associations between patient or disease characteristics and the development of second primary malignancies

Variable	*n*	*n* events / total events	HR (95% CI)	*P* value
Age	**154**	**-**	**1.06 (1.03 – 1.09)**	**<0.001**
***BRAF*^{V600E}**				
No	**81**	**4/18**	**REF**	
Yes	**73**	**14/18**	**3.17 (1.01 – 9.94)**	**0.048**
Multisystem disease				
No	97	9/18	REF	
Yes	57	9/18	2.29 (0.80 – 6.54)	0.12
First-line chemotherapy				
No	112	13/18	REF	
Yes	42	5/18	0.64 (0.19 – 2.09)	0.46

Note: 2/156 patients were not included in this analysis because of unknown first-line therapy (*n* = 1) or zero follow-up (*n* = 1). In total, 18/154 included patients had an event (SPM). Abbreviations: *n*, number; HR, hazards ratio; REF, reference; SPM, second primary malignancy.

SUPPLEMENTARY TABLE 4. Standardized incidence ratios and excess absolute risks of second cancers among the overall cohort and molecular subgroups

Type	Observed cases	Expected cases	PY	SIR (95% CI)	EAR[$] / 10.000 PY	*P*
Overall cohort						
All second cancers (SPM)*	18	4.79	922	3.76 (2.23-5.94)	14.32	**<0.001**
Second hematologic cancers (SHM)	9	0.42	949	21.25 (9.72-40.33)	9.04	**<0.001**
***BRAF*^{V600E} positive subgroup**						
All second cancers (SPM)*	14	2.45	437	5.72 (3.13-9.60)	26.41	**<0.001**
Second hematologic cancers (SHM)	7	0.21	462	32.71 (13.15-67.39)	14.70	**<0.001**
***BRAF*^{V600E} negative subgroup**						
All second cancers (SPM)*	4	2.34	485	1.71 (0.47-4.37)	3.42	0.45
Second hematologic cancers (SHM)	2	0.21	487	9.54 (1.16-34.48)	3.68	**0.005**

Abbreviations: PY, person-years; SIR, standardized incidence ratio; CI, confidence interval; EAR, excess absolute risk; SPM, second primary malignancies; SHM, second hematologic malignancies. Symbols: * Excluding basal cell carcinomas of the skin; [$] Excess absolute risks were calculated as observed minus expected numbers of second cancers per 10.000 person-years of follow-up.

SUPPLEMENTARY TABLE 5. Additional malignancies in patients from our cohort

Patient	LCH	Additional malignancies							
		1st | time from LCH diagnosis (years)		2nd | time		3rd | time		4th | time	
		Hematologic							
1 | NL60	MS-RO+	MDS	| + 0.7						
2 | US142	MS-RO-	AML	| + 1.1						
3 | US159	P-LCH	AML	| + 3.5						
4 | US169	SS-MFB	MDS	| + 5.3						
5 | US28	MS-RO+	AML	| + 8.1						
6 | US177	MS-RO+	SMZL	| - 3.1	AML	| + 5.1				
7 | US184	SS-skin	MM	| + 1.4						
8 | NL3	SS-UFB	DLBCL	| + 3.6						
		Hematologic + Solid							
9 | US32	SS-skin	Endometrium (carcinoma)	| - 20.9	CMML	| + 3.2				
10 | NL23	P-LCH	Cervix (SCC)	| - 7.7	FL/DLBCL	| - 4.9	Skin (SCC)	| + 1.3	Skin (SCC)	| + 10.1
		Solid							
11 | NL19	MS-RO-	Testis (seminoma)	| - 25.9						
12 | NL14	SS-UFB	Bone (osteosarcoma)	| - 22.3						
13 | US170	SS-GI	Rectum (adenocarcinoma)	| - 12.6						
14 | US7	MS-RO+	Prostate (adenocarcinoma)	| - 7.3						
15 | US219	SS-skin	Skin (melanoma)	| - 5.1						
16 | NL24	MS-RO-	Retroperitoneum (IMT)	| - 5.0						
17 | US166	MS-RO+	Prostate (adenocarcinoma)	| - 4.5						
18 | US203	P-LCH	Pancreas (NET)	| - 3.8						
19 | NL4	P-LCH	Testis (seminoma)	| - 2.5						
20 | US214	SS-liver	Pancreas (adenocarcinoma)	| - 2.0						
21 | US194	P-LCH	Lung (NSCLC)	| - 1.0						
22 | US195	SS-UFB	Skin (SCC)	| - 0.5						
23 | US216	MS-RO-	Thyroid (PTC)	| + 0.1						
24 | NL33	P-LCH	Lung (NSCLC)	| + 0.8						
25 | NL71	MS-RO-	Bladder (UC)	| + 1.0						
26 | US173	MS-RO-	Skin (melanoma)	| + 2.2						
27 | NL30	MS-RO+	Lung (NSCLC)	| + 3.9						
28 | NL28	MS-RO-	Breast (invasive carcinoma NST)	| + 9.9						
29 | NL44	SS-UFB	Colon (adenocarcinoma)	| + 18.8						
30 | NL2	SS-UFB	Thyroid (PTC)	| + 19.6						

BRAF^{V600E} + LCH

Myeloid
Lymphoid
Solid

Abbreviations: MS, multisystem; RO, risk organ; p-LCH, single system pulmonary LCH; SS, single system; MFB, multifocal bone; UFB, unifocal bone; GI, gastrointestinal tract; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; SMZL, splenic marginal zone lymphoma; MM, multiple myeloma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; SCC, squamous cell carcinoma; IMT, inflammatory myofibroblastic tumor; NET, neuroendocrine tumor; NSCLC, non-small cell lung cancer; PTC, papillary thyroid carcinoma; UCC, urothelial carcinoma; NST, no special type.

SUPPLEMENTARY TABLE 6. Prior treatments of patients with second primary malignancies

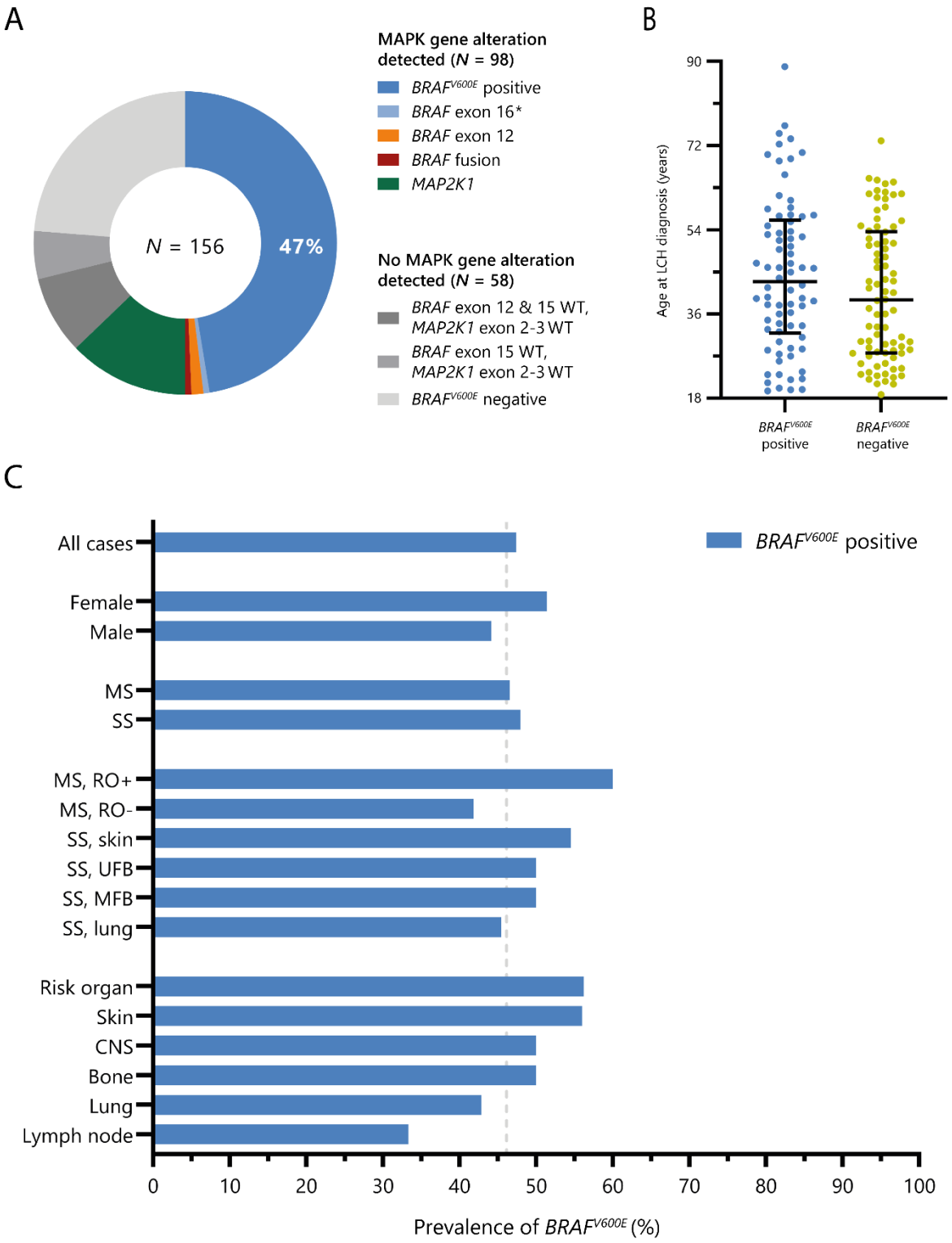
Patient	LCH extent at diagnosis	SPM	Time from LCH diagnosis (years)	Chemotherapy	Targeted therapy	Radiotherapy
1 | NL60	MS-RO+	MDS	| + 0.7	No	Yes: vemurafenib	No
2 | US142	MS-RO-	AML	| + 1.1	Yes: cladribine	No	No
9 | US32	SS-skin	CMML	| + 3.2	No	No	Yes
3 | US159	P-LCH	AML	| + 3.5	Yes: cladribine	No	No
6 | US177	MS-RO+	AML	| + 5.1	Yes: cladribine, vinblastine	No	Yes
4 | US169	SS-MFB	MDS	| + 5.3	Yes: vinblastine, cytarabine, hydrea	Yes: vemurafenib	Yes
5 | US28	MS-RO+	AML	| + 8.1	Yes: cladribine	No	No
7 | US184	SS-skin	MM	| + 1.4	Yes: hydrea	No	No
8 | NL3	SS-UFB	DLBCL	| + 3.6	No	No	No
23 | US216	MS-RO-	Thyroid (PTC)	| + 0.1	No	Yes: vemurafenib	No
24 | NL33	P-LCH	Lung (NSCLC)	| + 0.8	No	No	No
25 | NL71	MS-RO-	Bladder (UC)	| + 1.0	No	No	No
10 | NL23	P-LCH	Skin (SCC, 2x)	| + 1.3; + 10.1	Yes: CHOP, R-CVP and R-FC (for prior B-NHL)	Yes: rituximab	No
26 | US173	MS-RO-	Skin (melanoma)	| + 2.2	No	Yes: dabrafenib	No
27 | NL30	MS-RO+	Lung (NSCLC)	| + 3.9	No	No	No
28 | NL28	MS-RO-	Breast (invasive carcinoma NST)	| + 9.9	No	No	No
29 | NL44	SS-UFB	Colon (adenocarcinoma)	| + 18.8	No	No	No
30 | NL2	SS-UFB	Thyroid (PTC)	| + 19.6	Yes: vinblastine	No	No

New abbreviations: CHOP, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone; R-CVP, rituximab, cyclophosphamide, vincristine sulfate, and prednisone; R-FC, rituximab, fludarabine, and cyclophosphamide.

SUPPLEMENTARY TABLE 7. Articles included in the meta-analysis

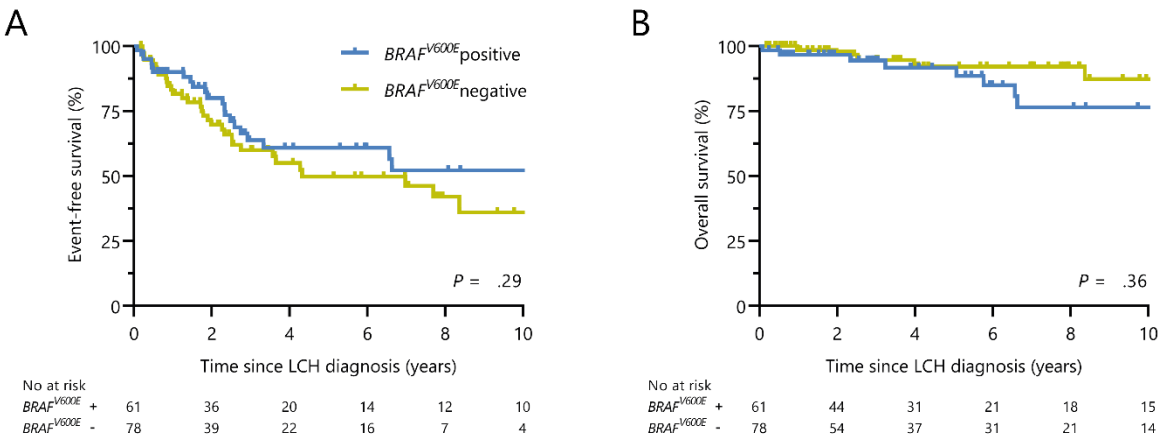
	References
Mutation frequency analysis	1,5-27
MS-SS analysis	6-8,11,14,15,18,20,22-25
MS-SS analysis, pulmonary SS LCH excluded	6-8,14,15,18,22,24,25
Event analysis	7,8,20,22,23

SUPPLEMENTARY FIGURE 1. Clinical features at LCH diagnosis by lesional *BRAF*^{V600E} status

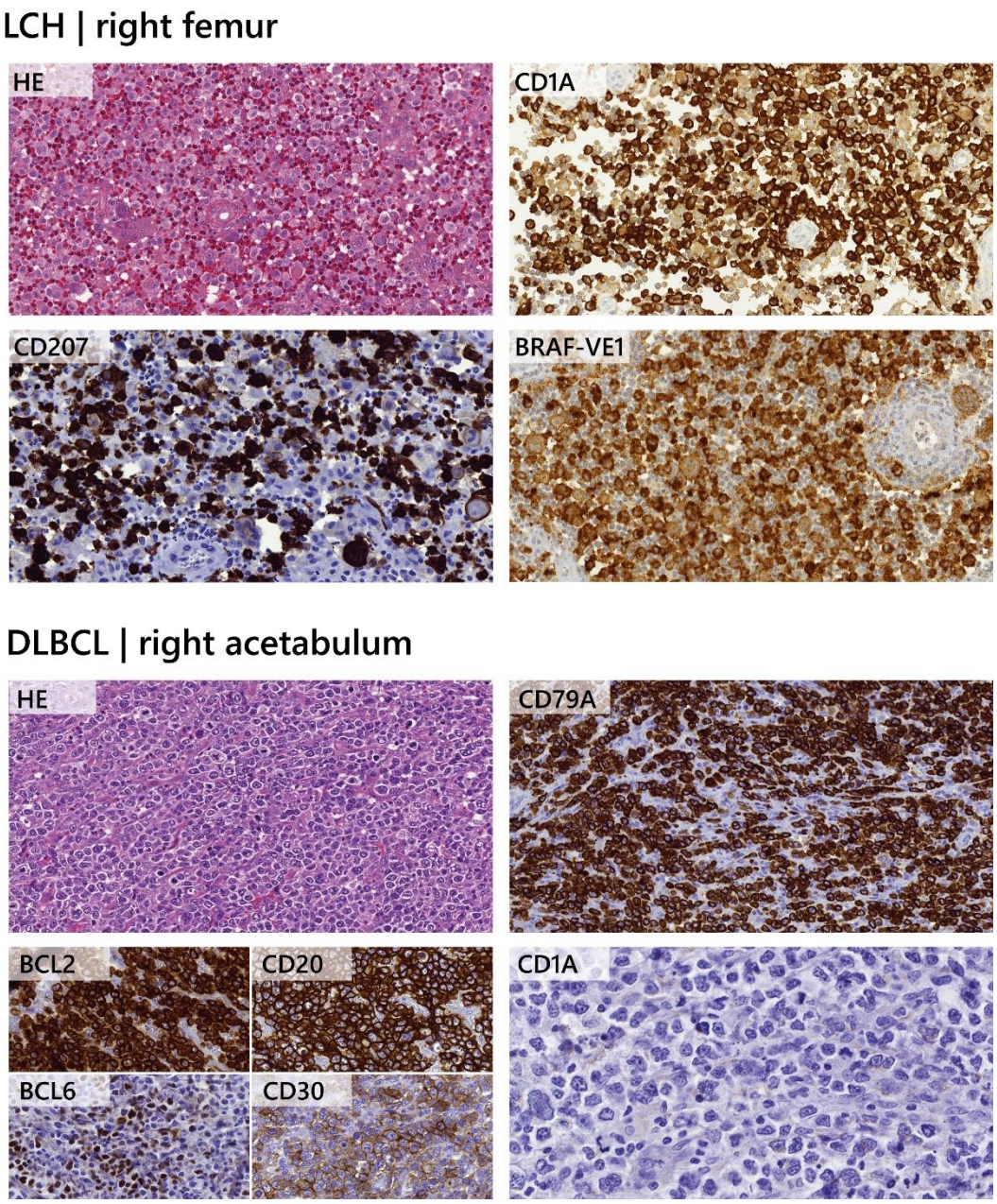


(A) Pie chart showing the mutational status of the 156 patients from our cohort. * Variant (*BRAF* p.R662K) with uncertain oncogenicity. **(B)** Dot plot showing age at diagnosis of patients with and without *BRAF*^{V600E}. Error bars depict medians with interquartile ranges. **(C)** Prevalence of *BRAF*^{V600E} among patients with specific clinical characteristics at diagnosis. Numbers of patients are provided in Table 1.

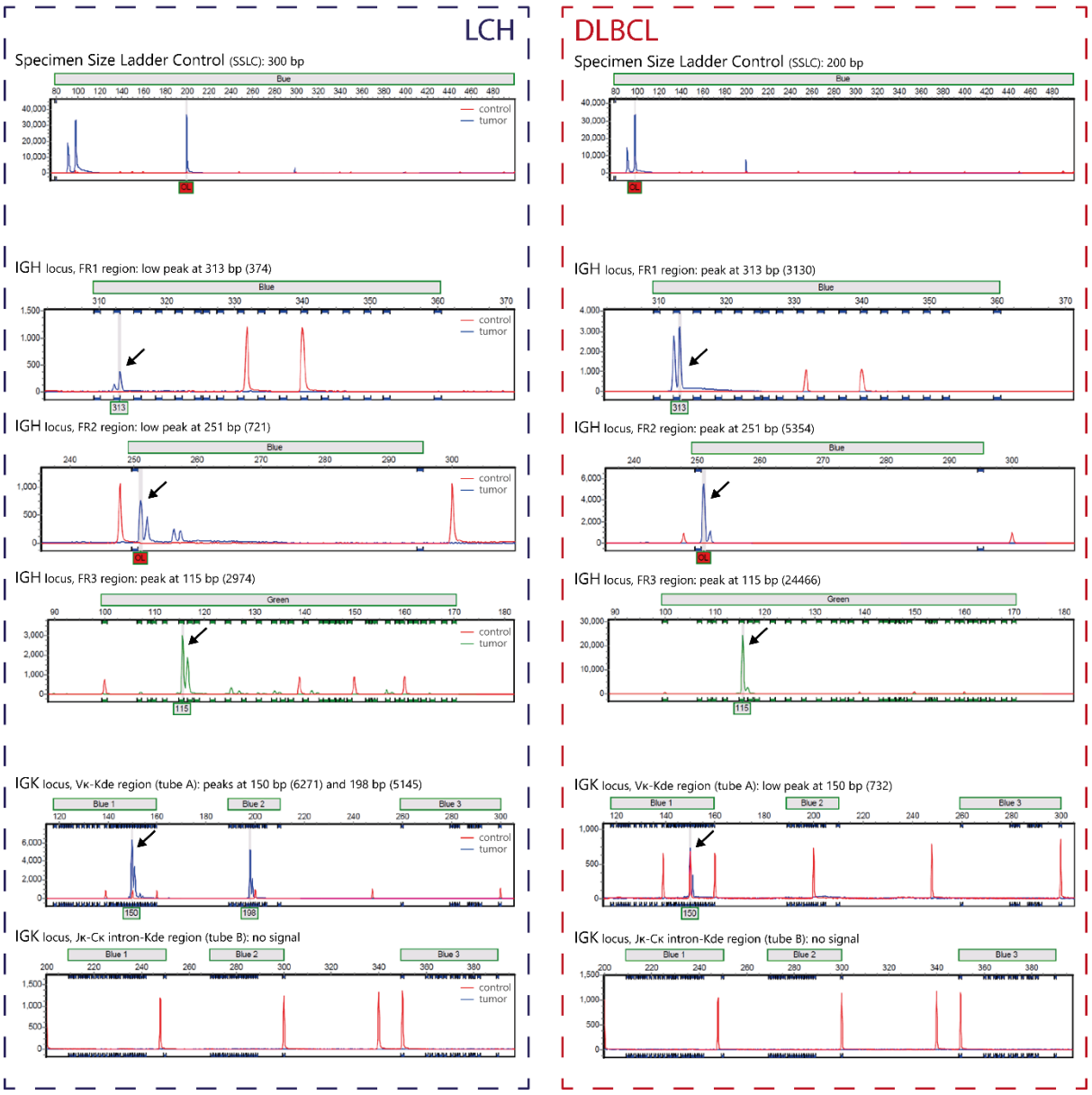
SUPPLEMENTARY FIGURE 2. Clinical outcomes of patients who did not receive targeted therapy



SUPPLEMENTARY FIGURE 3. Immunohistochemical findings in Case #8 who presented with a diffuse large B-cell lymphoma diagnosed 3.5 years after unifocal bone LCH



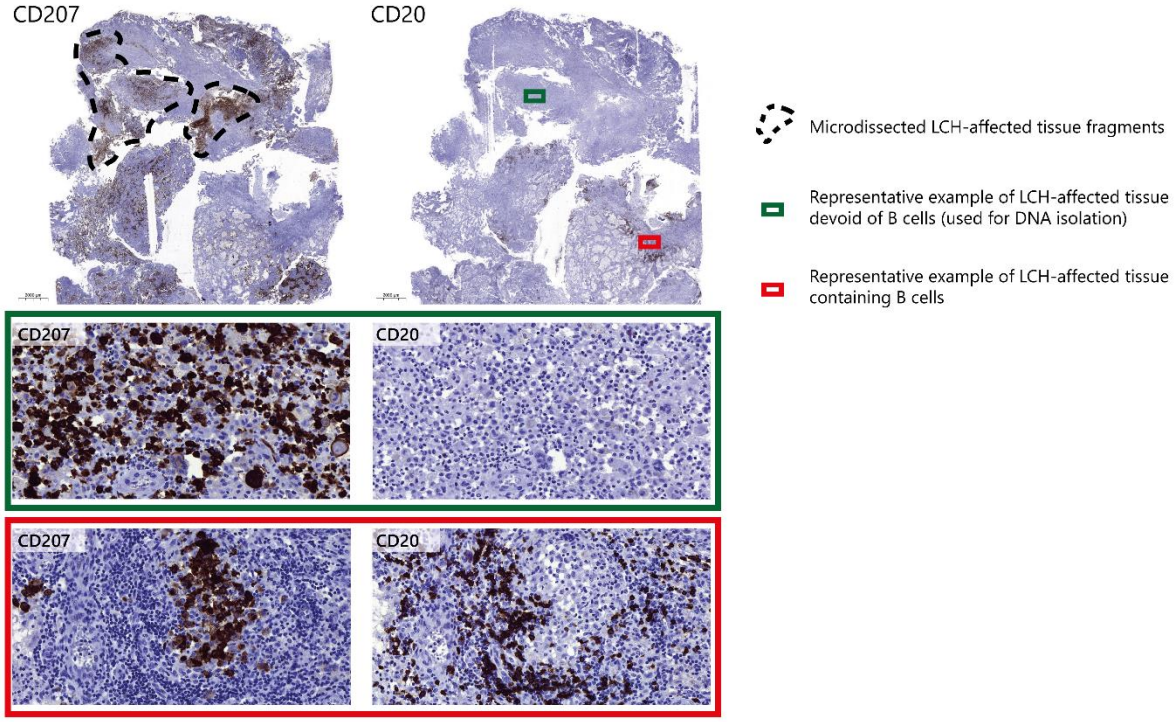
SUPPLEMENTARY FIGURE 4. PCR-based clonality assessment of immunoglobulin gene rearrangements in separate FFPE tissue samples of the LCH and DLBCL diagnosed in Case #8



Arrows depict the identical peaks identified in separate LCH and DLBCL samples by IGH and IGK gene rearrangement analysis using IdentiClone® kits. IdentiClone® kits are standardized multiplex polymerase chain reaction (PCR) assays. The IdentiClone® IGH + IGK B-cell Clonality Assay consists of tests that amplify the DNA between primers that target the conserved framework (FR) and joining (J) regions (IGH FR1, FR2, and FR3 region), the variable (V) and joining (J) regions (IGK tube A) and the variable, Jκ-Cκ intron, and K_{de} regions (IGK tube B). These conserved regions lie on either side of an area within the V-J region where genetic rearrangements occur during maturation of B lymphocytes. Each B cell has a single V-J rearrangement that is unique in both length and sequence. DNA from samples containing a clonal cell population harboring the same V-J rearrangement yield one or two prominent amplified products within a polyclonal background of different-sized products. Note that the primers that amplify the different FR regions, which are located at three distinct sections along the immunoglobulin heavy chain gene, produce a different size range of V-J products (https://invivoscribe.com/).

SUPPLEMENTARY FIGURE 5. Microdissected LCH-affected tissue fragments devoid of B cells used for NGS-based clonality assessment of immunoglobulin gene rearrangements in Case #8

A

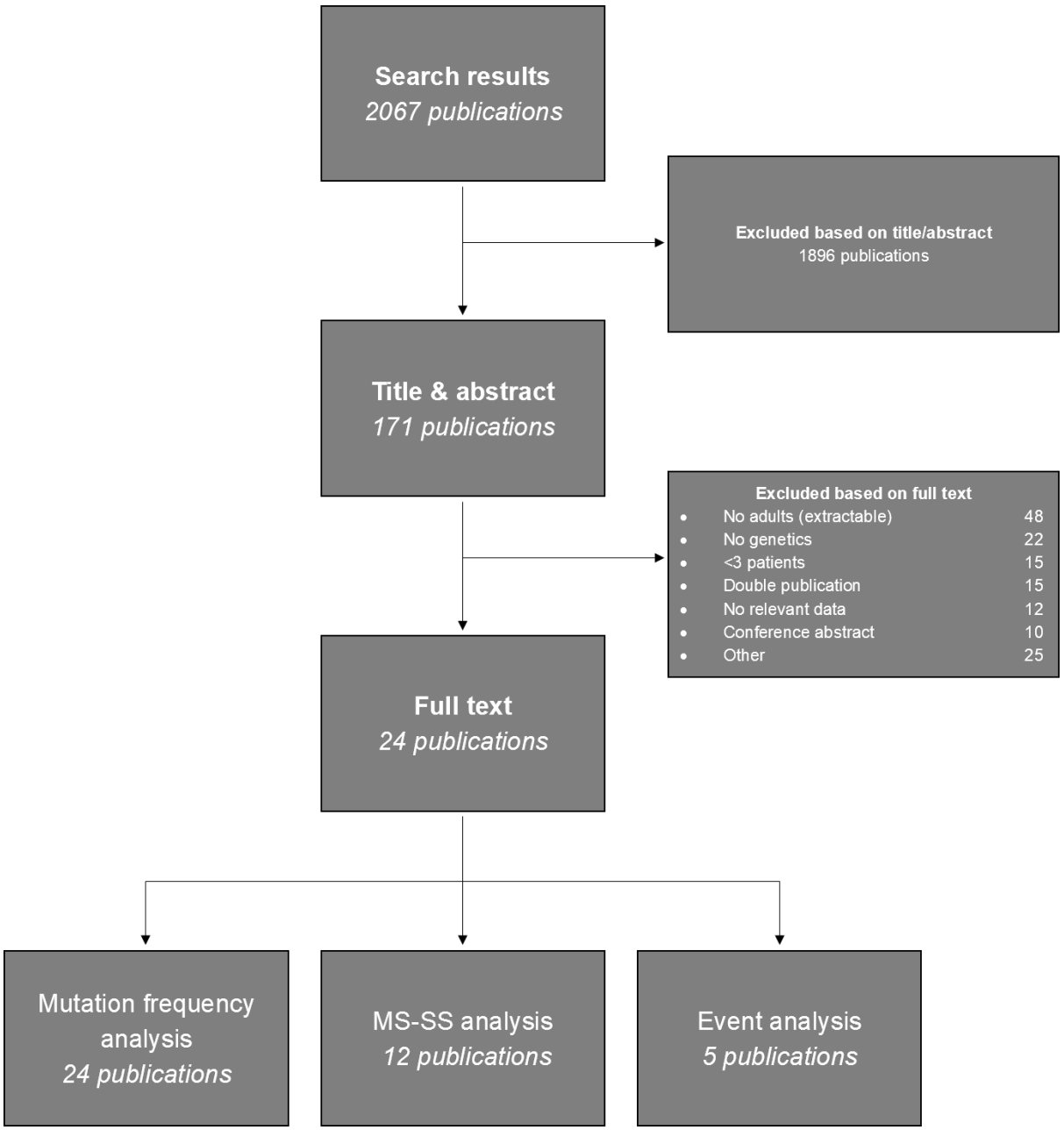


B

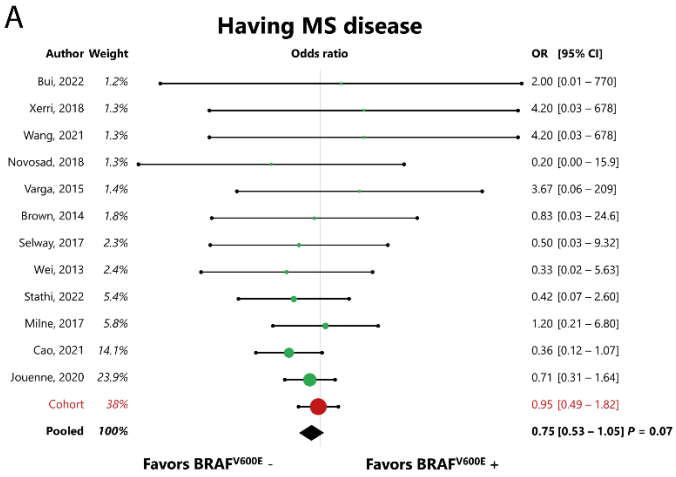
	LCH			DLBCL		
	Clonotype	Reads	Frequency	Clonotype	Reads	Frequency
IGHV-IGHJ FR2	V3-48 -1/14/-5 J4	4/4	100%	V3-48 -1/14/-5 J4	3284/3297	99.6%
IGHV-IGHJ FR3	V3-48 -1/14/-5 J4	1759/1873	93.9%	V3-48 -1/14/-5 J4	127851/130521	98.0%
IGHD-IGHJ	Multiple products	14122	-	Multiple products	9836	-
IGKV-IGKJ		QC fail			QC fail	

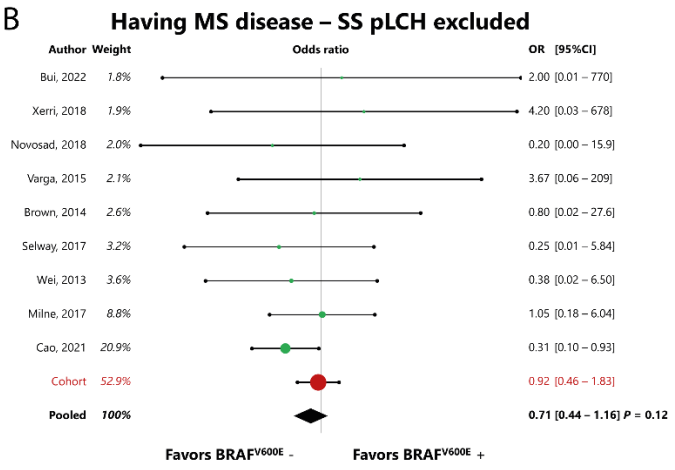
(A) Photomicrographs showing that the LCH-affected tissue sample of Case #8 consisted of tissue parts devoid of B cells (green rectangle), as well as tissue parts containing both $CD207^+$ histiocytes and $CD20^+$ B cells (red rectangle). Only tissue parts devoid of B cells, indicated by the dashed lines, were microdissected and used for DNA isolation. The isolated DNA was used for assessing IGH and IGK gene rearrangements using EuroClonality-NGS panels. **(B)** Overview of results of NGS-based clonality testing.

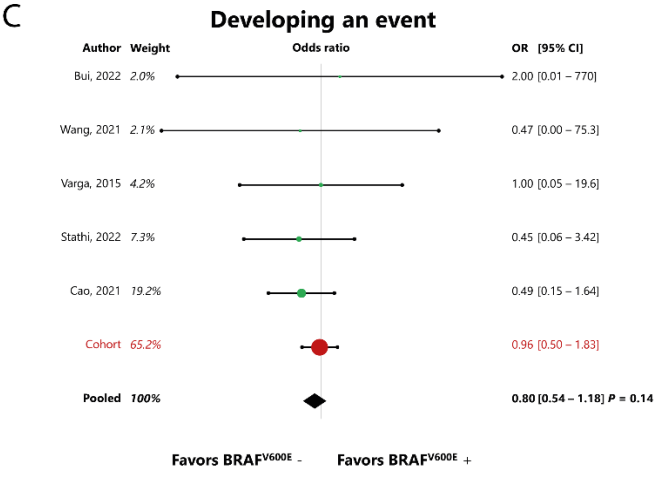
SUPPLEMENTARY FIGURE 6. CONSORT diagram showing the selection process of the systematic literature search



SUPPLEMENTARY FIGURE 7. Forrest plots regarding associations between *BRAF*^{V600E} and multisystem disease at diagnosis or developing an event







The current study cohort (Mayo Clinic + 3 Dutch academic centers) is indicated in red.

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