_BRAF_V600E in blood and brain and response to BRAFV600E inhibition suggest hematopoietic origin of neurodegeneration in LCH

Running Title: **CNS LCH: Hematopoietic origin and targets for therapy**

Kenneth McClain et al.

SUPPLEMENTAL METHODS

RNA purification and cDNA amplification

Total RNA was isolated normal brain, LCH lesions, LCH pituitary, LCH-ND brain, germinoma, and HLH samples according to the RNeasy FFPE Kit protocol (Qiagen, Valencia, CA). Complementary DNA (cDNA) amplification was performed with the Ovation FFPE WTA system, according to the manufacturer protocol (NuGen, San Carlos, CA).

Quantitative real-time PCR

Quantitative real-time PCR reactions were performed with standard methods**.** TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). Single-stranded cDNA was generated by RNA amplification as described above. Each reaction included 20 ng cDNA. TaqMan Fast Universal PCR Master Mix (Applied Biosystems, Foster City, CA) was used in 25 μl reactions in 96-well plates on a CFX96 Touch Real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA). Assays were performed in triplicate. Thermal cycling conditions were set at 10 minutes at 95˚C and then 40 cycles of 95˚C for 15 seconds and 60˚C for 1 minute.

TaqMan Probe Sets: (Applied Biosystems, Foster City, CA)

Gene Name	**TaqMan Probe**
GAPDH	Hs02758991_g1
CD3e	Hs01062241_m1
CD207 (langerin)	Hs00210453_m1
CD1a	Hs00381754_g1
OPN	Hs00959008_g1

Cellular BRAF*V600E qPCR Assay with Peripheral Blood Mononuclear Cells*

Genomic DNA (gDNA) was isolated from frozen PBMC using the QIAamp DNA micro protocols (QIAGEN, Valencia, CA). Purified gDNA was used in the *BRAF*V600E qPCR mutation assay (Somatic Mutation Assay for *BRAF*_476; Qiagen). *BRAF* mutation and reference primers were included in each reaction. Duplicate reactions were performed for each sample. All experiments were performed on a CFX96 Touch Real-time PCR detection system (Bio-Rad Laboratories). The means of C_t^{mut} and C_t^{ref} were calculated to determine $\Delta Ct = C_t^{mut(ave)} - Ct^{ref(ave).}$ The ΔCT was compared with a standard curve to estimate the percentage of cells with *BRAF*-V600E alleles. The standard curve was created by making 11 dilutions of gDNA isolated from A375 cell line (American Type Culture Collection (ATCC), Manassas, VA) with gDNA isolated from HEK293 cell line (ATCC). The ΔCT was plotted against the percentage of cells from A375/HEK293 cell pools (0.05%–100% *BRAF*V600E) from which the gDNA was isolated. For each LCH gDNA sample, the percentage of cells with the *BRAF*V600E mutation was then calculated based on the standard curve.

Extracellular BRAF*V600E qPCR Assay with Cerebral Spinal Fluid*

Whole Genome Amplification (WGA) was performed directly on CSF samples from LCH patients using the REPLI-g Midi Kit (Qiagen) per manufacturer's instructions. qRT-PCR was performed as described above on the amplified product. *BRAF*V600E: wild-type standard curves were created for amplified gDNA from 11 dilutions of amplified gDNA from A375 cells with amplified gDNA from HEK293 cells. The amplified product was also tested by the QX100 Droplet Digital PCR (ddPCR™) system (Bio-Rad Laboratories) per manufacturer's instructions.

Immunohistochemistry

FFPE tissue sections (3-5μm) were de-paraffinization and rehydration through a graded alcohol series, antigen retrieval was performed on FFPE slides using as indicated below. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 10 minutes. Sections were incubated with primary antibodies at 4°C for overnight. Following incubation with secondary antibodies at room temperature for 1 hour, immunoreactivity were detected as outlined below. Slides were counter-stained with hematoxylin before imaged with Olympus BX53 microscope (10X/ 0.30 ∞/-/FN26.7, 20X / 0.50 ∞/0.17/FN26.5, 40X / 0.75 ∞/0.17/FN26.6) fitted with a DP26 camera.

Antibody (Source)	Cat#	Clone	Dilution	Antigen Retrieval	Detection
CD163 (Novocastra)	CD163-L-CE	10D6	1:200	uCC1 mild (Ventana proprietary reagents)	iView DAB(Ventana proprietary reagents)
CD33 (Leica)	NCL-L-CD33	PWS44	1:100	uCC1 mild (Ventana proprietary reagents)	OptiView DAB(Ventana proprietary reagents)
CD14 (abcam)		7	1:10	uCC1 mild (Ventana proprietary reagents)	Optiview DAB(Ventana proprietary reagents)

BRAF V600E (Ventana Medical Systems)	790-4855	VE1	Pre-dilute	uCC1 standard (Ventana proprietary reagents)	OptiView DAB(Ventana proprietary reagents)
CD3 (Abcam)	ab699	PS1	1:50	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
CD207 (Santa Cruz Biotechnology)	sc-33767	N/A	1:50	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
VE-1 (New East Biosciences)	26039	N/A	1:50	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
OPN (Santa Cruz Biotechnology)	sc21742	Akm2A1	1:50	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
S100B (Millipore)	ABN59	N/A	1:200	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
MCP-1(Abcam)	ab9669	N/A	1:50	Target Retrieval Solution (Dako)	DAB(Innovex Biosciences)
P2yp12 (Sigma)	HPA014518	N/A	1:1000	Target Retrieval Solution (Dako)	NovaRED Vector Labs SK-4800

Immunoblot Analysis

LCH and BT patient biopsy cell suspension were lysed in buffer containing 25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40 and 5% glycerol containing Halt protease inhibitor cocktail (Thermo SCIENTIFIC, Rockford, IL), phosphatase inhibitor cocktails 2 and 3 (Sigma-Aldrich, St Louis, MO) and 1 mM sodium orthovanadate (Sigma-Aldrich). Ten µg of whole cell lysates were resolved on a Criterion TGX 10% 4-20% (OPN) and Tris-Tricine 16%(S100B) gel (Bio-Rad, Hercules, CA), transferred to an Immobilon PVDF membrane (Millipore), and probed with antibodies recognizing OPN (sc21742, Santa Cruz Biotechnology, Dallas, TX) and S100B (ABN59, Millipore). The blots were subsequently stripped and re-probed with GAPDH antibody (Cell Signaling Technologies) to confirm equal loading.

SUPPLEMENTAL TABLES

Supplemental Table 1. CSF analytes.

Supplemental Table 2. Significant CSF biomarkers

Table 3A. Significant CSF biomarkers: Discovery and validation - LCH vs BT and ALL

Table 3B. Significant CSF biomarkers: Full dataset - LCH vs HLH

Table 3C. Significant CSF biomarkers: Full dataset - LCH subjects with CNS tumor vs BT

Table 3D. Significant CSF biomarkers: Full dataset – LCH-ND vs Non-LCH-ND

Supplemental Table 3. Identification of *BRAF*V600E in peripheral blood fractions of subjects with LCH CNS lesions

Supplemental Table 4. Clinical courses of patients treated with *BRAF*V600E inhibition.

Radiologic and clinical responses are reported as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Radiologic response is reported as both interval change from previous study as well as cumulative change relative to initiation of MAPK inhibitor therapy. Clinical response is reported as interval change and objectively measured using the ataxia rating score. Intrathecal methotrexate/hydrocortisone (IT MTX/HCT). (A) LCH0008. (B) LCH0035. (C) LCH0060. (D) LCH0004.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Study patients and samples

A. Venn diagram identifies the inclusion of subjects across the different experiments in this study. "CSF" indicates CSF proteomics and CSF extracellular BRAF-V600E genomic DNA qPCR testing. "PBMC Blood (Pre/post-chemotherapy)" indicates PBMC from blood specimens were used for cellular genomic DNA qPCR testing at various timepoints relative to chemotherapy. "PBMC Blood (BRAFi therapy)" indicates PBMC from blood specimens were used for cellular genomic DNA qPCR testing at various timepoints relative to BRAF inhibitor therapy. "Biopsy" indicates subjects with biopsy specimens used in this study.

B. Flow-chart of CSF specimens in proteomics studies, BRAF-V600E qPCR studies, or both. For CSF studies, 1 CSF specimen was used for each subject.

C. Flow-chart of patients and PBMC specimens used in BRAF-V600E qPCR studies. (P) indicates the number of patients. (S) indicates the number of samples. The "Treatment" column is color coded with respect to the Figure in which the results from the specimens are illustrated. Venn diagram indicates the PBMC sample groups (pre-chemotherapy; post-chemotherapy with systemic disease; post-chemotherapy without systemic disease) to which subjects contributed specimens.

Supplemental Figure 2. Serial analysis of *BRAF*V600E allele in PBMC.

Evaluation for *BRAF*V600E allele in PBMC in LCH-ND patients across serial blood specimens in patients with proven *BRAF*V600E+ lesions and/or detectable *BRAF*V600E+ in PBMC at any point. Datapoints describe radiologic and clinical details of LCH-ND

along with treatment details over time (years on x-axis) where PMBC were evaluated for *BRAF*V600E (% on y-axis).

Supplemental Table 1: Detailed Analyte Information

Millipore Kit	Catalog Number	Dilution Factor	Analytes
Human Cytokine/Chemokine I	HCYTMAG-60K-PX38	1:2	EGF, FGF-2, Eotaxin, TGF-α, G-CSF, Flt-3L, GM-CSF, Fractalkine, IFN-α2, INF-γ, GRO, IL-10, MCP-3, IL-12p40, MDC, IL-12p70, IL-13, IL-15, SCD40-L, IL-17, IL-1RA, sIL-2RA, IL-1α, IL-9, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6 (2), IL-7, IL-8 (2), IP-10, MCP-1, MIP-1α, MIP-1β, TNF-α, TNF-β, VEGF
Human Cytokine/Chemokine Panel II	HCP2MAG-62K-PX23	1:2	Eotaxin-2, MCP-2, BCA-1, MCP-4, I-309, IL-16, TARC, 6CKine, Eotaxin-3, LIF, TPO, SCF (2), TSLP, IL-33, IL-20, IL-21, IL-23, TRAIL (2), CTACK, SDF-1α+β, ENA-78, MIP-1d, IL-28A
Human Cytokine/Chemokine Panel III	HCYP3MAG-63K	1:2	CXCL6/GCP-2, CXCL11/I-TAC, CCL19/MIP-3β, CCL20/MIP-3α, XCL1/Lymphotactin, IL-11, IL-29/IFN-γ1, CXCL9/MIG, M-CSF
Human Circulating Cancer Panel 1	HCCBP1MAG-58K	1:2	AFP, Total PSA, CA15-3, CA19-9, MIF, TRAIL, Leptin, Free PSA, IL-6, sFasL, CEA, CA125, IL-8, HGF, SDF1α+β, sFas, TNF-α (2), Prolactin, SCF, CYFRA 21-1, OPN, FGF-2 (2), β-HCG, HE4, TGF-α (2), VEGF (2)
Human Neurodegenerative Disease Panel 1	HNDG1MAG-36K	1:400	Apolipoprotein AI, Apolipoprotein CIII, Apolipoprotein E, Complement C3, α2-Macroglobulin, Prealbumin, Complement Factor H
Human Neurodegenerative Disease Panel 2	HNDG2MAG-36K	1:20	CRP, α1-Antitrypsin, PEDF, SAP, MIP-4, Complement C4
Human Neurodegenerative Disease Panel 3	HNDG3MAG-36K	Neat	BDNF, sVCAM-1, sICAM-1, MPO, Cathepsin D, PDGF-AA, PDGF-AB/BB, RANTES, PAI-1 (Total), NCAM
Human Neurodegenerative Disease Panel 4	HNDG4MAG-36K	1:3	S100B, Ab40, Ab42, GDNF, sRAGE

Supplemental Table 2

A. CSF Biomarkers: Discovery and Validation (LCH vs. ALL vs. BT)

	Analytes	Log Transformed Mean Concentration (pg/ml) (±2 SD)			p-value	LCH vs. ALL		LCH vs. BT	
		LCH	ALL	BT		p-value	Significant	p-value	Significant
Discovery	S100B	9.3 ± 0.7	9.0 ± 0.9	11.2 ± 1.9	8.65E-05	3.26E-01	No	4.00E-03	Yes
	OPN	17.0 ± 1.3	15.6 ± 0.8	14.9 ± 1.5	1.98E-04	2.54E-03	Yes	5.90E-04	Yes
Validation	S100B	8.9 ± 0.8	8.8 ± 0.7	10.9 ± 1.5	9.20E-06	9.09E-01	No	8.33E-04	Yes
	OPN	17.9 ± 1.2	15.6 ± 0.9	15.5 ± 1.9	3.59E-05	9.96E-06	Yes	1.17E-03	Yes

B. CSF Biomarkers: Full Dataset (LCH vs. HLH)

Analytes	Log Transformed Mean Concentration (pg/ml) (±2 SD)		Log	Fold	p-value
	LCH	HLH	Fold Change	Change	
OPN	17.0 ± 1.4	15.3 ± 1.4	1.69	3.23	1.77E-03
IL-12 (p40)	2.3 ± 0.5	2.9 ± 0.7	-0.56	0.68	9.57E-03
Fractalkine	6.9 ± 0.6	7.5 ± 1.0	-0.64	0.64	1.22E-02
IL-1RA	2.9 ± 0.5	3.5 ± 0.9	-0.66	0.63	4.04E-03
IL-7	2.1 ± 0.6	2.8 ± 0.6	-0.69	0.62	2.08E-03
IFN-a2	3.0 ± 0.6	3.8 ± 0.9	-0.79	0.58	1.94E-03
IL-1a	2.0 ± 0.6	2.9 ± 1.2	-0.93	0.53	1.32E-03
IL-8	5.1 ± 0.8	6.1 ± 1.3	-1.08	0.47	1.74E-03
PAI-1 (total)	9.1 ± 0.6	10.1 ± 1.2	-1.09	0.47	2.05E-04
IL-8 (2)	4.3 ± 0.8	5.5 ± 1.2	-1.20	0.43	4.45E-04
GRO	4.1 ± 0.8	5.4 ± 1.5	-1.27	0.41	5.19E-04
BCA-1	1.1 ± 0.6	2.6 ± 2.5	-1.54	0.34	8.42E-04
IL-10	1.7 ± 0.5	3.3 ± 2.2	-1.57	0.34	1.38E-04
RANTES	1.7 ± 1.1	3.5 ± 2.1	-1.72	0.30	1.23E-03
G-CSF	4.2 ± 1.2	6.4 ± 1.2	-2.18	0.22	1.00E-05
CXCL11/I-TAC	2.4 ± 0.6	4.9 ± 2.1	-2.43	0.19	1.00E-07
IP-10	8.9 ± 1.4	11.6 ± 2.8	-2.64	0.16	1.30E-04
CRP	13.2 ± 3.5	17.0 ± 2.5	-3.89	0.07	2.63E-03

C. CSF Biomarkers: Full Dataset (LCH (CNS Tumor) vs. BT)

Analytes	Log Transformed Mean Concentration (pg/ml) (±2 SD)		Log	Fold	p-value
	LCH (CNS Tumor)	BT	Fold Change	Change	
OPN	16.7 ± 1.3	15.2 ± 1.7	1.55	2.94	9.49E-04
S100B	9.5 ± 0.8	11.1 ± 1.7	-1.52	0.35	2.93E-04

D. CSF Biomarkers: Full Dataset (LCH (ND+) vs. ND CTL)

Analytes	Log Transformed Mean Concentration (pg/ml) (±2 SD)		Log	Fold	p-value
	LCH (ND+)	ND CTL	Fold Change	Change	
OPN	17.5 ± 1.3	16.0 ± 1.3	1.44	2.71	2.58E-05
S100B	9.0 ± 0.8	9.9 ± 0.9	-0.86	0.55	8.60E-05

Supplemental Table 3: Identification of *BRAF*V600E in peripheral blood fractions of subjects with LCH CNS lesions

Sample	Total Events	%Viability	Sort Purity	T-Cells CD3	B-Cells CD19	Macs CD64	Mono/NK CD16	DC Precursor CD11c	Myeloid DC CD11c + CD14	Lin+ DR-	Negative Fraction CD11c-ve CD14-ve
LCH0001	71000	89%	>85%	-	-	-	**0.79**	**0.05**	-	-	-
LCH0004	62000	82%	>85%	**0.08**	-	-	-	-	-	**0.58**	-
LCH0016	63000	82%	>85%	**0.70**	-	-	-	-	-	**0.14**	-
LCH0021	54000	80%	>85%	**0.09**	-	**0.07**	**0.18**	-	**0.12**	-	-
LCH0034	62000	80%	>85%	-	-	-	**0.82**	-	-	-	-
LCH0035	74000	84%	>85%	-	-	-	-	-	**0.09**	-	-
LCH0040	72000	81%	>85%	-	-	**0.46**	**0.55**	**0.78**	**1.33**	-	-
LCH0044	61000	87%	>85%	-	-	-	-	-	**0.05**	-	-
LCH0061	81000	87%	>85%	-	-	-	-	-	-	-	-
LCH0063	60000	82%	>85%	-	-	-	**0.07**	-	**0.02**	-	-

Supplemental Table 4

A. Clinical Response Form: LCH0008

Start	End	Clinical Event	Sites of Lesions	ND	Therapy	Response Lesion	Response ND (MRI) - Interval	Response ND (MRI) - Cumulative	Response ND (clinical) - Interval	ARS	PBMC *BRAF*V600E (%)
4/2014	10/2014	• New diagnosis	Scalp and diaper rash, colon, bone marrow, liver	No	clofarabine, methylprednisolone,	CR	NA	NA	NA	0-0	NA
10/2014	9/2015	• Transition to maintenance therapy	None	No	mercaptopurine methotrexate (PO)	CR	NA	NA	NA	0-0	NA
11/2015	12/2015	• Pneumonia • Thrombocytopenia • Splenomegaly	None	No	antibiotics and supportive care	CR	NA	NA	NA	0-0	NA
12/2015	1/2016	• Relapse #1 • Diagnosis of HLH	Spleen, liver, bone marrow	No	rituximab, methylprednisolone	PR (HLH) PD (LCH)	NA	NA	NA	0-0	NA
1/2016	2/2016	• Splenomegaly with refractory • Thrombocytopenia • LCH-associated HLH • Abnormal behavior	Spleen, liver, bone marrow, CSF, CNS	CT Clinical	splenectomy HLH-directed therapy etoposide dexamethasone	SD (HLH) PD (LCH)	NA	NA	PD	26/47-42/42	12.9
2/2016	3/2016	• Seizure • Somnolent • Abnormal behavior • Decreased strength • Worsening ataxia • Intellectual decline • Speech regressed • Disseminated fungal infection	Brain, CSF, liver, bone marrow	MRI Clinical	cytarabine, cladribine	PR	PD	NA	PD	42/45	0.08
3/2016	5/2016	• Started dabrafenib • Improved strength • Improved speech • Improved behavior • Intellectual decline resolved • Decreased ataxia	Brain, CNS, bone marrow, liver	MRI Clinical	dabrafenib 25 mg BID (96 mg/m^2/day)	PR	PD	PD	PR	30-42/47	0.11
5/2016	6/2016	• Improved strength • Improved speech • Improved behavior • Slightly worsened ARS (due to unrelated fall w/ femur fracture) • Sitting normally, unassisted • Improving liver function	Brain, CSF, liver, bone marrow	MRI Clinical	dabrafenib	PR	PR	PR	PR	29/55	0.99
6/2016	7/2017	• Neurologic symptoms resolved	None	MRI	dabrafenib	CR	SD	PR	CR	0	0.83

Radiologic Response (Corresponding to Figure 5)

Initially, there is moderate hyperintense FLAIR signal in bilateral dentate and peridentate regions, dorsal pons, bilateral putamen, globus pallidi, bilateral paramedian thalami and mesial temporal regions. Two years later, there has been interval progression of the neurodegenerative disease on MR with increased hyperintense FLAIR signal and extent of the involvement in the aforementioned areas as well as new development of signal abnormality in bilateral cerebellum white matter centrally. At 15-month follow-up after the treatment with BRAF inhibitor, there is significant improvement of signal abnormality in the bilateral cerebellum, mesial temporal lobes, basal ganglia and thalami.

Supplemental Table 4

B. Clinical Response Form: LCH0035

Start	End	Clinical Event	Sites of Lesions	ND	Therapy	Response Lesion	Response ND (MRI) - Interval	Response ND (MRI) - Cumulative	Response ND (clinical) - Interval	ARS	PBMC *BRAF*V600E (%)
1/2007	3/2008	• New diagnosis	Scalp rash Temporal bone	No	vinblastine prednisone	CR	NA	NA	NA	0-0	NA
12/2008	1/2009	• Diabetes insipidus	Pituitary	MRI	None	SD	PD	NA	NA	0-0	NA
1/2009	11/2012	• Relapse#1	Parietal	MRI	curettage	CR	SD	NA	NA	0-0	0-0.017
11/2012	7/2013	• Relapse#2	Mandible	MRI	cytarabine cladribine	CR	SD	NA	NA	0-0	0.018-0.04
8/2013	9/2013	• Onset of clinical ND	None	MRI Clinical	None	CR	PD	NA	PD	0-16	NA
9/2013	12/2013	• Relapse #3 • Worsening ataxia	Spinal cord	MRI Clinical	clofarabine IT MTX/HCT dexamethasone	PD	PD	NA	PD	16-90	0-0.015
12/2013	2/2014	• Wheelchair bound • Worsening ataxia • Intellectual decline	Same	MRI Clinical	clofarabine IT MTX/HCT dexamethasone	PD	PD	NA	PD	90-94	0
2/2014	4/2014	• Improved strength and coordination	Same	MRI Clinical	clofarabine IT MTX/HCT dexamethasone	SD	PR	NA	PR	94-90	0
4/2014	7/2014	• Improved strength • Ataxia unchanged	Same	MRI Clinical	vemurafenib 480 mg BID (807 mg/m^2/day)	PR	PD	PD	PD	90-88	0
7/2014	11/2014	• Improved speech • Improved bladder function • Regained ability to sit	Spinal cord normal Brain slightly improved	MRI Clinical	vemurafenib 720 mg BID (1083 mg/m^2/day)	PR	PR	PR	NA	88-80	0
11/2014	12/2014	• No change	Same	MRI Clinical	vemurafenib rituximab	SD	NA	PR	SD	80-80	0
12/2014	4/2015	• Improved strength • Improved speech • Decreased ataxia	Same	MRI Clinical	vemurafenib	SD	PR	PR	PR	80-75	0
4/2015	7/2015	• Uveitis • Declining strength • Abnormal behavior	Same	MRI Clinical	discontinued vemurafenib	PR	NA	PR	PD	75-80	0
7/2015	1/2016	• Improved strength • Improved speech • Decreased ataxia	Spinal cord resolved CNS persists	MRI Clinical	restart vemurafenib 240 mg BID (343 mg/m^2/day) increased to 480 mg AM/240 mg PM (518 mg/m^2/day)	CR	NA	PR	PR	80-58	0
1/2016	7/2017	• Improved strength • Improved speech • Decreased ataxia • Hip surgery for avascular necrosis – wheelchair bound	Same	MRI Clinical	hydroxyurea	CR	SD	PR	PR	50-43	0

Radiologic Response (Corresponding to Figure 5)

Initially, there is moderate hyperintense FLAIR signal in bilateral dentate and peridentate regions, dorsal pons, bilateral putamen, globus pallidi, bilateral paramedian thalami and mesial temporal regions. Two years later, there has been interval progression of the neurodegenerative disease on MR with increased hyperintense FLAIR signal and extent of the involvement in the aforementioned areas as well as new development of signal abnormality in bilateral cerebellum white matter centrally. At 15-month follow-up after the treatment with BRAF inhibitor, there is significant improvement of signal abnormality in the bilateral cerebellum, mesial temporal lobes, basal ganglia and thalami.

Supplemental Table 4

C. Clinical Response Form: LCH0060

Start	End	Clinical Event	Sites of Lesions	ND	Therapy	Response Lesion	Response ND (MRI) - Interval	Response ND (MRI) - Cumulative	Response ND (clinical) - Interval	ARS	PBMC *BRAF*V600E *(%)*
1/2005	1/2008	• New diagnosis	Humerus	No	curettage	CR	NA	NA	NA	0-0	NA
1/2008	1/2011	• Relapse #1	Tibia	No	curettage	CR	NA	NA	NA	0-0	NA
1/2011	10/2012	• Ataxia, learning problems, emotional lability	None	Clinical	None	CR	NA	NA	PD	10-14	NA
10/2012	12/2013	• Improved neurologic symptoms	None	MRI Clinical	cytarabine	CR	PR	NA	PR	14-8	0-0.013
6/2014	12/2014	• Worsening neurologic symptoms	None	MRI Clinical	off treatment 6 months	CR	PD	NA	PD	8-16	0
1/2015	7/2015	• Hydrocephalus, ataxia worse	None	MRI Clinical	cytarabine	CR	PD	NA	PD	16-20	0
8/2015	10/2015	• Stable neurologic symptoms	None	MRI Clinical	vemurafenib 240 mg AM/360 mg PM ($536\ mg/m^2/day$)	CR	PR	PR	SD	20-20	Detectable (<0.01%)
10/2015	3/2016	• Improved speech and balance	None	MRI Clinical	vemurafenib	CR	PR	PR	PR	20-9	0.01
3/2016	5/2017	• Speech and ataxia worse • Not compliant with medications	None	MRI Clinical	vemurafenib	CR	SD	PR	PD	19-29	0

Radiologic Response (Corresponding to Figure 5)

Initially, there is mild hyperintense FLAIR signal in bilateral posterior centrum semiovale and bilateral central cerebellar white matter. There has been worsening of signal abnormality in bilateral cerebellum on the follow up 4 years later. After 8-months of treatment with BRAF inhibitor, there is significant improvement of signal abnormality in the bilateral cerebellum.

Supplemental Table 4

D. Clinical Response Form: LCH0004

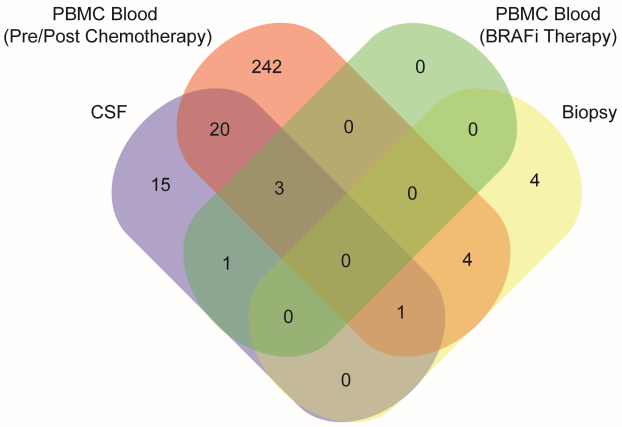
Start	End	Clinical Event	Sites of Lesions	ND	Therapy	Response Lesion	Response ND (MRI) - Interval	Response ND (MRI) - Cumulative	Response ND (clinical) - Interval	ARS	PBMC *BRAF*V600E *(%)*
1/1997	1/1998	• Presentation of LCH	Lung, skin, intestine, femur, occiput	No	vinblastine, prednisone, methotrexate	CR	NA	NA	NA	0-0	NA
1/1999	1/2002	• Relapse #1	Frontal skull	No	vinblastine, prednisone	CR	NA	NA	NA	0-0	NA
10/2003	6/2005	• Relapse #2 • Ataxia, dysarthria, dysmetria	None	MRI Clinical	None	CR	NA	NA	NA	0-4	NA
7/2005	3/2006	• Worsening neurologic symptoms	None	MRI Clinical	Vincristine cytarabine	CR	SD	NA	PD	28-32	NA
7/2006	6/2007	• Worsening neurologic symptoms	None	MRI Clinical	IVIG	CR	SD	NA	PD	32-60	0.12-0.05
7/2007	1/2008	• Worsening neurologic symptoms	None	MRI Clinical	sirolimus, IVIG	CR	PR	NA	SD	74-68	NA
3/2008	10/2008	• Hydrocephalus • Worsening neurologic symptoms	None	MRI Clinical	ventriculoperitoneal shunt	CR	SD	NA	PR	68	NA
8/2009	7/2010	• Ataxia, speech improved	None	MRI Clinical	imatinib, sirolimus, IVIG	CR	PR	NA	PR	68-55	0.03
8/2010	12/2011	• Worse ataxia, inappropriate behavior, confined to wheel chair	None	MRI Clinical	imatinib, sirolimus, IVIG	CR	PD	NA	PD	55-80	NA
1/2012	5/2013	• Dysphagia, speech and spasticity worse	None	MRI Clinical	imatinib, sirolimus, IVIG	CR	SD	NA	PD	80-90	0.08
6/2013	3/2015	• Speech, swallowing, spasticity better	None	MRI Clinical	rituximab, sirolimus, IVIG	CR	PD	NA	PR	90-85	0.12-0.24
6/2015	7/2015	• Trial of vemurafenib initiated	None	MRI Clinical	vemurafenib 480 mg BID (545 mg/m^2/day), sirolimus, IVIG	CR	SD	SD	SD	85-85	NA
7/2015	8/2015	• Joint pain, rash	None	MRI Clinical	discontinued vemurafenib	CR	PD	PD	PD	85-92	0.03-0.06
8/2015	2/2016	• Speech, choking, tremors and spasticity worsen • Reaction to rituximab	None	MRI Clinical	rituximab, hydroxyurea	CR	NA	PD	PD	92-95	0.03-0.24
2/2016	3/2016	• Worsening neurologic symptoms	None	MRI Clinical	hydroxyurea	CR	NA	PD	PD	95-96	0.04
3/2016	1/2017	• Rituximab increased to 555 mg/m^2 • Modest improvement in speech • Decreased choking • Increased strength	None	MRI Clinical	dabrafenib 100 mg BID (118 mg/m^2/day), rituximab	CR	PD	PD	SD	96-93	0.04-0.11
1/2017	3/2017	• Rash • Ptosis and periorbital swelling • Dabrafenib stopped • Worsened neurologic symptoms	None	MRI Clinical	rituximab	CR	SD	PD	PD	93-96	0.18
3/2017	5/2017	• Speech, choking, tremors and spasticity stable • Rituximab reaction	None	MRI Clinical	dabrafenib 75 mg BID (87 mg/m^2/day), trametinib 2 mg daily (1.2 mg/m^2/day), rituximab	CR	PD	PD	SD	93-92	0.34
5/2017	7/2017	• Folliculitis • Choking improved • Speech worse	None	MRI Clinical	dabrafenib, trametinib, obinutuzumab	CR	NA	PD	PD	92-94	NA

Radiologic Response (Corresponding to Figure 5)

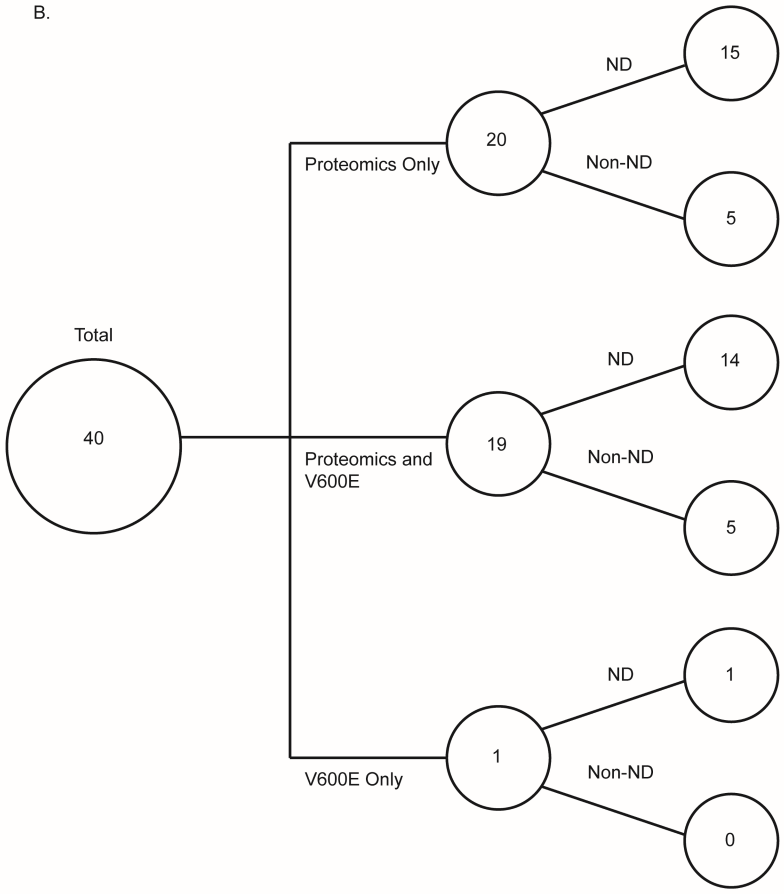
There is persistent hyperintense FLAIR signal in the bilateral dentate, peridentate regions, bilateral central white matter of the cerebellum and periventricular white matter after 10 years follow-up. Borderline volume loss in bilateral basal ganglia is also noted. Twenty-five months following BRAF inhibitor treatment, there is subtle progression of the signal abnormality in the bilateral cerebellum and increased mineralization of the globous pallida.

Supplemental Figure 1.

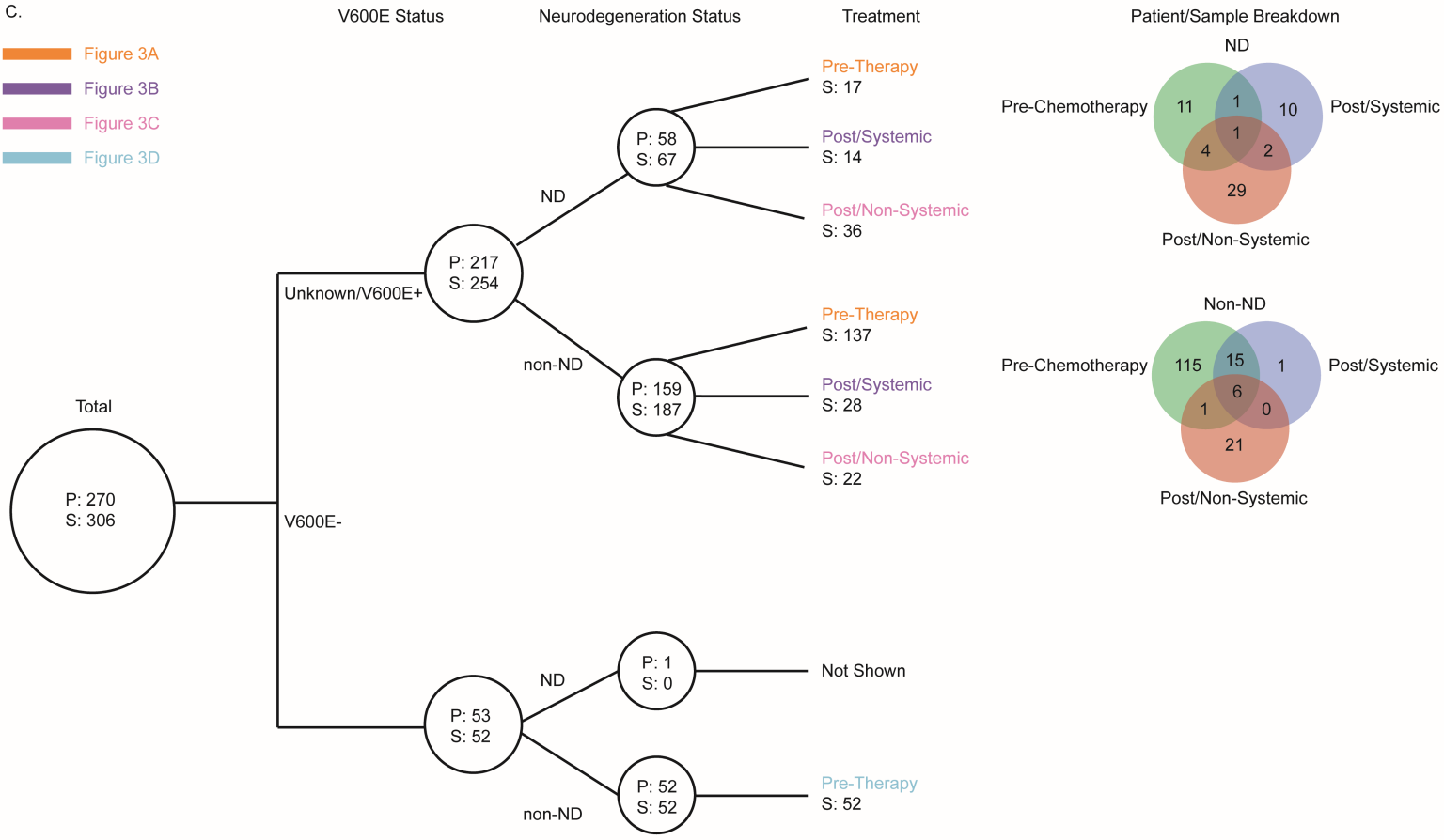
A.



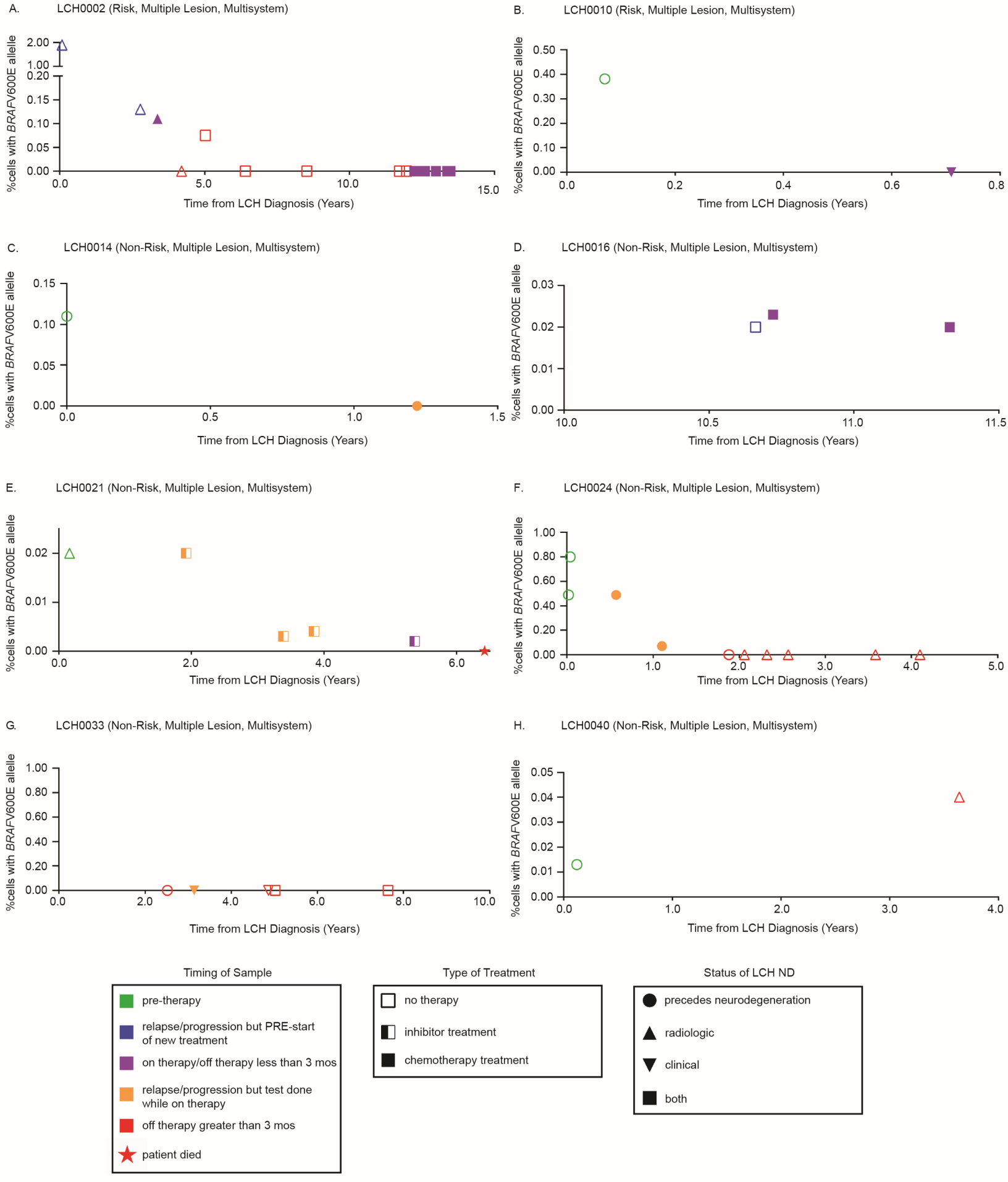
B.



C.



Supplemental Figure 2.



Supplemental Figure 2.