

BRAF V600E-positive cells as molecular markers of bone marrow disease in pediatric Langerhans cell histiocytosis

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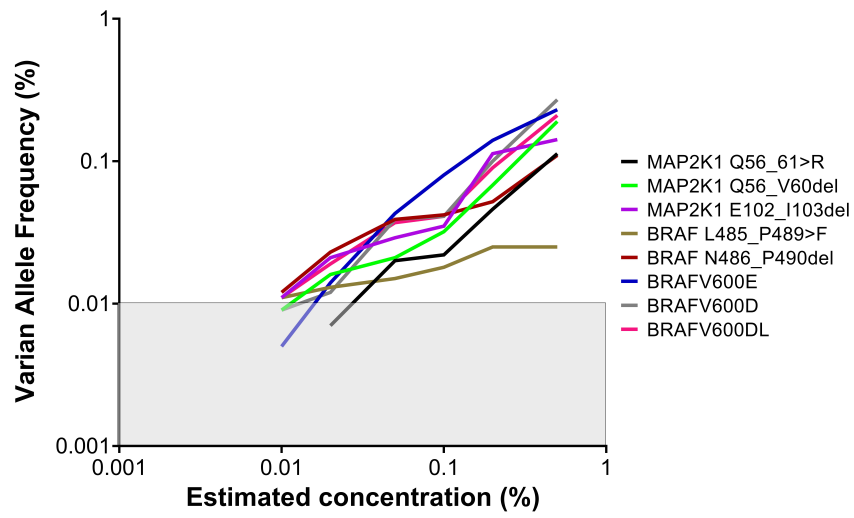
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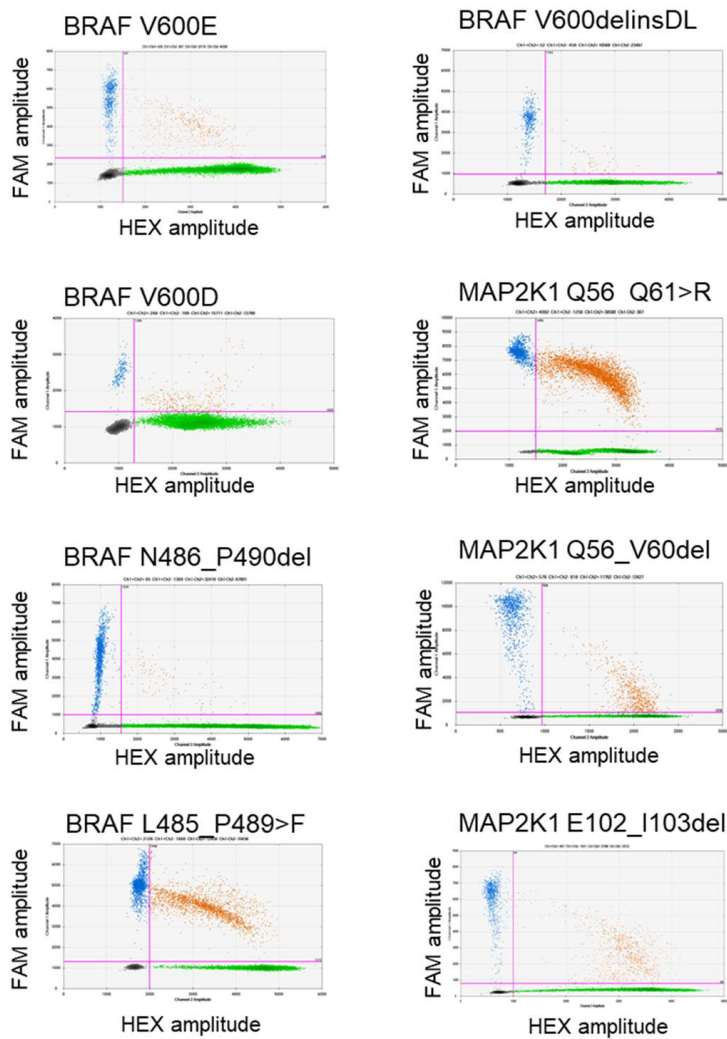
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Supplementary Figure S1

A



B



Supplementary Figure S1. Mutational analysis by allele-specific droplet digital PCR (ddPCR) to detect various variants including *BRAF*^{V600E}. (A) **The detection sensitivity of various variants by ddPCR.** To validate the detection sensitivity of various variants by ddPCR, mutation-positive samples were diluted and prepared with predicted mutant allele frequencies of 0.5%, 0.2 %, 0.1%, 0.02%, and 0.01%, respectively, for analysis. Although the detection sensitivity varied depending on the custom-made primers, the detection was possible down to 0.01%. (B) **Representative two-dimensional ddPCR plots using commercially available primers for *BRAF*^{V600E} variant and custom-designed primers for other variants.** The two-dimensional plots of ddPCR are representative of formalin-fixed, paraffin-embedded (FFPE) tumor samples positive for the somatic mutations of *BRAF* V600E, *BRAF* V600D, *BRAF* V600delinsDL, *BRAF* N486-P490del, *BRAF* L485_P489del, *MAP2K1* Q56_Q61>R, *MAP2K1* Q56_V60del, and *MAP2K1* E102_I103del. Fluorescein (FAM)-positive droplets indicate the presence of mutant DNA (blue); hexachlorofluorescein (HEX)-positive droplets indicate the presence of wild-type DNA (green); double-positive droplets indicate the presence of both mutant and wild-type DNA (orange).

Supplementary Table S1. Target regions and primer sequences used for amplicon targeted sequencing

Primer sequence for amplicon targeted sequencing		Annealing temperature, °C
Target amplicon	Adaper/locus-specific sequence	
BRAF exon 12		
Forward	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/AAAATTGAAATGACACTTGGAGTAACAAT-3'	60
Reverse	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/CAGGAGCTAATAAAAATAACTTCTTCTCTG-3'	
BRAF exon 15		
Forward	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/TTTCCTTTACTTACTACACC-3'	56
Reverse	5'-GTCCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/TAATCAGTGGAAAAATAGCC-3'	
MAP2K1 exon2		
Forward	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/CCCCACTTTGGAACAGGAC-3'	59
Reverse	5'-GTCCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/TGTTAATCAAGGCAAACTCACC-3'	
MAP2K1 exon3		
Forward	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG/CCTTCCTCCCTTTTCTTTCA-3'	61
Reverse	5'-GTCCTCGTGGGCTCGGAGATGTGTATAAGAGACAG/GACTGAGAGGGTGTACATA-3'	
Primer and probe sequences used for droplet digital PCR		
Target amplicon	Primer/probe sequence	Annealing temperature, °C
BRAF L485_P489>F: c.1455_1469delGGTGCTGTCACATTC, p.Leu485_Pro490delinsPhe		
Forward	5'-TTGAAGCCTTGTAAGTCTG-3'	57
Reverse	5'-ATTGAAATGACACTTGGAGTAACA-3'	
Probe for wild type	5'-HEX/AGCACCTACACC-3'	
Probe for mutant	5'-FAM/AAATGTTTACACCTC-3'	
BRAF N486_P490del: c.1457_1471del, p.Asn486_Pro490del		
Forward	5'-TTGAAGCCTTGTAAGTCTG-3'	58
Reverse	5'-ATTGAAATGACACTTGGAGTAACA-3'	
Probe for wild type	5'-HEX/AGGTGTAGGTGC-3'	
Probe for mutant	5'-FAM/AGGTGTCAACATT-3'	
BRAF V600E: c.1799T>A, p.Val600Glu		
Forward	5'-GAGTAATTCACACAAGCTCACC-3'	55
Reverse	5'-TGAATGACACTTGGAGTAACA-3'	
Probe for wild type	5'-HEX/TAGGTGCTGTCAC-3'	
Probe for mutant	5'-FAM/CTGAGGTTTCAACATT-3'	
BRAF V600D: c.1799_1800delTGinsAT, p.Val600Asp		
Forward	5'-TTCATGAAGACCTCACAGTAA-3'	56
Reverse	5'-GCCTCAATTCCTACCATCCA-3'	
Probe for wild type	5'-HEX/AGCTACAGTGAATCTCG-3'	
Probe for mutant	5'-FAM/AGCTACAGATAATCTCG-3'	
BRAF V600delinsDL: c.1798_1799insATT, p.Val600delinsAspLeu		
Forward	5'-TTCATGAAGACCTCACAGTAA-3'	56
Reverse	5'-GCCTCAATTCCTACCATCCA-3'	
Probe for wild type	5'-HEX/AGCTACAGTGAATCTCG-3'	
Probe for mutant	5'-FAM/TACAGATTTGAAATC-3'	
MAP2K1 Q56_G61>R: c.167_181delAGAAGCAGAAGGTGG, p.Gln56_Gly61delinsArg		
Forward	5'-TGGAGGAGCTAGAGCTTGAT-3'	59
Reverse	5'-AGGCTTGTGGGAGACCT-3'	
Probe for wild type	5'-HEX/CTTACCCAGAAG-3'	
Probe for mutant	5'-FAM/CTTACGGGAGAA-3'	
MAP2K1 Q56_V60del: c.165_179delCCAGAAGCAGAAGGT, p.Gln56_Val60del		
Forward	5'-TGGAGGAGCTAGAGCTTGAT-3'	58
Reverse	5'-AGGCTTGTGGGAGACCT-3'	
Probe for wild type	5'-HEX/AGAAGCAGAAGGTGG-3'	
Probe for mutant	5'-FAM/TTCTTACCCGAGAAGTGA-3'	
MAP2K1 E102_I103del: c.303_308delGGAGAT, p.Glu102_Ile103del		
Forward	5'-CTTCCACCTTTCTCCAGCTA-3'	56
Reverse	5'-CCACGATGTACGGAGAGTT-3'	
Probe for wild type	5'-HEX/TTCATCTGGAGA-3'	
Probe for mutant	5'-FAM/TTCATCTCAAACC-3'	

Supplementary Table S2. Characteristics of 59 Langerhans cell histiocytosis patients whose tumor samples were subjected to targeted sequencing

Case	Age, Y	Gender	Disease type	Disease status	Disease burden	Progression/Recurrence	Gene alteration	NGS variant allele frequency, %	ddPCR variant allele frequency, %	
P1	1.8	M	MSRO-	new ly diagnosed	Multifocal bone, orbit, mastoid antrum, skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	2.30	14.48
P2	5.4	F	MSRO-	new ly diagnosed	Localized bone, pituitary gland	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	24.00	23.46
P3	16.1	F	SS	new ly diagnosed	Hypothalamus	neg	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	5.30	3.96
P4	0.8	F	MSRO+	new ly diagnosed	Spleen, liver, ear canal, lymph nodes	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.46	3.76
P5	0.9	M	MSRO-	new ly diagnosed	Multifocal bone, skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	34.77	33.57
P6	1.7	F	MSRO-	new ly diagnosed	Localized bone, skin, ear canal	relapse	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	15.84	9.02
P7	2.3	M	MFB	new ly diagnosed	Multifocal bone	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	16.67	26.92
P8	1.7	F	MFB	new ly diagnosed	Multifocal bone	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	20.76	10.31
P9	0.8	F	MSRO-	new ly diagnosed	Localized bone, lymph nodes	neg	<i>MAP2K1 Q56_Q61>R</i>	c.167_181delAAGAAGCA GAAAGTGG, p.Gln56_Gly61delinsArg	6.80	13.60
P10	0.4	M	MSRO-	new ly diagnosed	Localized bone, thymus	neg	<i>BRAFV600D</i>	c.1799_1800delTGinsAT, p.Val600Asp	11.00	6.80
P11	1.4	M	MSRO-	new ly diagnosed	Localized bone, skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.50	5.89
P12	0.6	F	MSRO-	new ly diagnosed	Localized bone, liver, spleen	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	17.00	19.39
P13	5.4	F	MSRO-	relapsed	Localized bone, soft tissue	relapse	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	14.90	3.59
P14	1.7	F	MFB	new ly diagnosed	Multifocal bone	neg	<i>BRAFV600delinsDL</i>	c.1798_1799insATT, p.Val600delinsAspLeu	13.00	16.40
P15	0.4	F	MSRO-	new ly diagnosed	Localized bone, skin	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.40	4.77
P16	1.5	F	MSRO+	new ly diagnosed	Localized bone, bone marrow, skin	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	29.30	29.72
P17	0.1	M	MSRO-	new ly diagnosed	Lungs, thymus, skin	neg	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	19.12	7.20
P18	7.2	M	MFB	new ly diagnosed	Multifocal bone	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	7.29	3.20
P19	6.9	F	MFB	new ly diagnosed	Multifocal bone	neg	<i>BRAFV600D</i>	c.1799_1800delTGinsAT, p.Val600Asp	3.54	1.66
P20	0.1	M	MSRO+	new ly diagnosed	Gut, oral mucosa, bone marrow, skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.12	11.20
P21	0.3	F	MSRO+	new ly diagnosed	Localized bone, lymph nodes, liver, spleen, skin, bone marrow	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	23.00	18.70
P22	8.1	M	SS	new ly diagnosed	Sphenoidal sinus	neg	<i>MAP2K1 Q56_V60del</i>	c.165_179delCCA GAAAGCAAGGT, p.Gln56_Val60del	7.78	8.50
P23	2	F	MSRO+	relapsed	Spleen, skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	18.70	4.70
P24	0.8	M	MFB	new ly diagnosed	Multifocal bone	relapse	<i>MAP2K1 Q56_G61>R</i>	c.167_181delAAGAAGCA GAAAGTGG, p.Gln56_Gly61delinsArg	6.20	9.30
P25	1.4	F	SS	new ly diagnosed	Localized bone	neg	<i>MAP2K1 E102_I103del</i>	c.303_308delGGAGAT, p.Glu102_Ile103del	2.48	9.14
P26	14.1	M	SS	relapsed	Localized bone	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	24.46	22.70
P27	7.8	M	MSRO-	relapsed	Localized bone, lymph nodes	neg	<i>BRAFex12SE</i>	c.1511_1517+2dupTACTCAGGT	2.33	NA
P28	2.5	F	MSRO-	new ly diagnosed	Multifocal bone, ear canal, mastoid antrum	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.25	3.81
P29	5.4	M	SS	new ly diagnosed	Localized bone	neg	<i>MAP2K1 Q56_G61>R</i>	c.167_181delAAGAAGCA GAAAGTGG, p.Gln56_Gly61delinsArg	11.77	4.67
P30	10	M	MFB	new ly diagnosed	Multifocal bone	neg	<i>MAP2K1 E102_I103del</i>	c.303_308delGGAGAT, p.Glu102_Ile103del	20.37	14.76
P31	1.5	M	MSRO+	relapsed	Liver, spleen, bone marrow, skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	12.65	13.50
P32	0.7	M	MSRO+	new ly diagnosed	Localized bone, spleen, liver, lungs, mediastinum, skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	13.06	10.90
P33	1.4	M	MSRO+	new ly diagnosed	Localized bone, liver, bone marrow, lymph node, skin	neg	<i>MAP2K1 F53_Q58>L</i>	c.159_173delTCTTACCCA GAAAGCA, p.Phe53_Gln58delinsLeu	3.79	NA
P34	5.3	M	MFB	new ly diagnosed	Multifocal bone	neg	NA	NA	NA	NA
P35	4.6	F	MSRO+	new ly diagnosed	Liver, spleen, bone marrow, skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	14.60	16.10
P36	0.8	M	MSRO-	new ly diagnosed	Thymus, liver, bone marrow, skin	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	8.74	5.47
P37	3	F	MSRO-	new ly diagnosed	Mastoid antrum, pituitary gland	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	9.20	8.40
P38	1.2	M	MFB	new ly diagnosed	Multifocal bone	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	33.78	33.80
P39	0.4	M	MSRO+	new ly diagnosed	Localized bone, bone marrow, spleen, bone, lymph node, ear canal skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	10.00	8.70
P40	0.3	F	MSRO-	new ly diagnosed	Multifocal bone, pituitary gland, ear canal	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	29.00	23.40
P41	0.5	M	MSRO+	new ly diagnosed	Guts, liver, spleen, bone marrow, skin	progression	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	8.00	6.10
P42	1.3	F	SS	new ly diagnosed	Localized bone	relapse	NA	NA	NA	NA
P43	1.5	F	SS	new ly diagnosed	Ear canal, mastoid antrum	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	9.70	13.24
P44	7.7	M	SS	new ly diagnosed	Cervical lymph nodes	relapse	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	15.00	19.10
P45	3.7	M	SS	new ly diagnosed	Ear canal, mastoid antrum	neg	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	3.20	9.09
P46	0.2	M	MSRO-	new ly diagnosed	Skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	6.30	2.94
P47	9.0	M	SS	new ly diagnosed	Localized bone	neg	<i>MAP2K1 F53_Q58>L</i>	c.159_173delTCTTACCCA GAAAGCA, p.Phe53_Gln58delinsLeu	7.40	NA
P48	9.0	M	SS	new ly diagnosed	Localized bone	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.50	4.08
P49	1.1	M	MSRO-	new ly diagnosed	Jaw, skin	relapse	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	4.20	3.24
P50	0.5	M	SS	new ly diagnosed	Skin	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	2.80	11.15
P51	1.5	F	MSRO-	new ly diagnosed	Oral mucosa, maxillary sinus, skin	neg	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	23.00	13.00
P52	8.0	M	MSRO-	new ly diagnosed	Localized bone	NA	<i>BRAFN486_P490>K</i>	c.1458_1472delGTGACAGCACTAC, Asn486_Thr491delinsLys	9.51	11.17
P53	14.0	M	SS	new ly diagnosed	Localized bone	NA	NA	NA	NA	NA
P54	0.9	M	SS	new ly diagnosed	Localized bone	NA	NA	NA	NA	NA
P55	6.0	M	SS	new ly diagnosed	Skin	NA	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	10.96	11.34
P56	16.0	M	MFB	new ly diagnosed	Multifocal bone	NA	<i>BRAFV600E</i>	c.1799T>A, p.Val600Glu	14.10	17.23
P57	4.0	F	SS	new ly diagnosed	Localized bone	NA	NA	NA	NA	NA
P58	4.0	F	SS	new ly diagnosed	Localized bone	NA	<i>BRAFN486_P490del</i>	c.1457_1471del, p.Asn486_Pro490del	5.36	11.00
P59	5.0	M	SS	new ly diagnosed	Localized bone	NA	<i>BRFL485_P489>F</i>	c.1455_1469delGGTCTGTCACTTC, p.Leu485_Pro490delinsPhe	21.67	22.10

M, male; F, female; MSRO-, multi-system disease; MSRO+, multi-system disease with risk organ involvement; MFB, multi-focal bone disease; SS, single-system disease; NA, not applicable; NGS, next-generation sequencing