Spectrum of histiocytic neoplasms associated with diverse haematological malignancies bearing the same oncogenic mutation

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Supplementary Material

Figure S1. Coverage of various genes by the different NGS panels used to analyse the affected tissue specimens of the three cases presented in this study. Genes depicted in green were targeted by the NGS panel used to analyse the tissue specimen(s) affected by the specified neoplasm (represented by a column). Genes depicted in red were not targeted by the NGS panel. Only genes that were targeted by NGS in two or more cases are shown.

Table S1. Available PALGA diagnostic terms for the various histiocytic disorders, used to retrieve relevant pathology reports from the Dutch Pathology Registry. Some diagnostic terms are given in Dutch.

Disorder	Diagnostic terms	Corresponding PALGA-codes
LCH	• langerhanse cel histiocytose • histiocytose X • eosinofiel granuloom • granuloma eosinophilicum • hand schuller christian • abt letterer siwe • letterer siwe	M97511 M77860 M97520 M97223
JXG	• juveniel xanthogranuloom • xanthogranuloma juveniel • xanthogranuloma juvenile • xanthoendothelioom • naevoxanthoendothelioom	M55380
RH	• reticulohistiocytoom • reticulohistiocytair granuloom	M77880
XD	• xanthoma disseminatum	M55350
HS	• ml true histiocytic • ml true histiocytic lu • malignant lymphoma true histiocytic lu • histiosarcoom • histiocytosarcoom • histiocytair sarcoom	M97233
MH	• histiocytaire medullaire reticulose • maligne histiocytose • histiocytaire maligniteit • maligniteit histiocytaire origine	M97203 T05120M95993
IDCS	• interdigiterend dendritisch cel sarcoom • metastase interdigiterend dendritisch cel sarcoom	M88083 M88086
ECD	• erdheim chester; • morbus erdheim chester	M97551
RDD	• rosai-dorfman ziekte	M75680
BPDCN	• blastaire plasmacytoide dendritische cel neoplasie • blastair NK-cel lymfoom • aggressieve blastaire NK-cel leukemie	M98753 M97503

Abbreviation	Disorder
LCH	Langerhans cell histiocytosis
JXG	juvenile xanthogranuloma
RH	reticulohistiocytosis
XD	xanthoma disseminatum
HS	histiocytic sarcoma
MH	malignant histiocytosis
IDCS	interdigitating dendritic cell sarcoma
ECD	Erdheim-Chester disease
RDD	Rosai-Dorfman disease
BPDCN	blastic plasmacytoid dendritic cell neoplasm

TABLE S2. Extended table of reported patients with histiocytic neoplasms associated with additional haematological malignancies bearing the same genetic alteration(s) as demonstrated by DNA sequencing and/or DNA methylation profiling.

Nr	P/A	Age	G	Histiocytic classification[1]	Histiocytic neoplasm	Additional haematological malignancy	Analysis method	Shared genetic alteration(s)	Shared *TCR* or Ig rearrangements	Unique genetic alteration(s)	Interval	Concurrently present	Outcome (follow-up)[2]	Reference
1	P	8y	F	L group	**LCH**	**T-ALL**	1. WES; 2. SNP array analysis	1. ***KRAS*** p.G12V, ***NOTCH1*** p.Q2501X, ***CREBBP*** p.R2066H, ***BRAF*** p.G464V and 14 VUS; 2. Homozygous CN loss at 9p21.3, including ***CDKN2A/2B***	N/A	T-ALL: *GATA3* p.A287T and 10 VUS; LCH: 18 VUS (& the shared ***NOTCH1*** , ***CREBBP*** and ***BRAF*** mutations were enriched)	LCH diagnosis 8 months **after** T-ALL diagnosis	Possibly	Died (15m)	Kato M, *Br J Haematol* 2016.
2	P	7y	M	L group	**LCH**	**T-ALL**	Direct sequencing of PCR products	***NOTCH1*** p.Q2405X[3]	Yes (TCRγ)	T-ALL: ***NOTCH1*** p.I1719T	LCH diagnosis 22 months **after** T-ALL diagnosis	Possibly	Died (3m)	Yokokawa Y, *Genes Chromosomes Cancer* 2015. & Kato M, *Br J Haematol* 2016.
3	A	44y	M	L group	**LCH**	**HCL**	BRAF-VE1 (LCH) & pyrosequencing (HCL)	***BRAF*** p.V600E	Yes (not further specified)	N/A	LCH diagnosis 9 months **after** HCL diagnosis	Yes	N/A	Loghavi S, *Blood* 2017.
4	A	46y	F	L group	**LCH**	**HL**	NGS (LCH) & Idylla ctBRAF PCR (HL)	***BRAF*** p.V600E	N/A	N/A	LCH diagnosis at least 1 year **after** HL diagnosis[4]	No	N/A	Haefliger S, *Ann Hematol* 2018.
5	A	78y	F	L group	**LCH**	**CMML**	Taqman PCR, HRM analysis, pyrosequencing & BRAF-VE1	***BRAF*** p.V600E	NR	N/A	LCH diagnosis 4 years **before** CMML diagnosis	Yes	Died (3y)	Konstantinou MP, *J Eur Acad Dermatol Venereol* 2020.
6	A	63y	M	L group	**LCH**	**AML NOS**	NGS	***ASXL1*** , *IDH2* and *STAG2* mutations	NR	LCH: ***BRAF*** p.V600E	LCH diagnosis **after** AML diagnosis	Yes[5]	Alive (N/A)	Khurana S, *JAMA Dermatol* 2020.
7	A	45y	M	L group	**LCH**	**AML NOS**	1. Karyotyping (AML) & FISH (both); 2. ARMS-PCR	1. Trisomy 8; 2. ***KRAS*** p.A146T	NR	LCH: ***BRAF*** p.V600E	**Concurrent**	Yes	N/A	Wang X, *Blood* 2019 (ASH abstract).
8	A	70y	F	L group	**LCH**	**PMF**	RQ-PCR	***JAK2*** p.V617F	NR	N/A	LCH diagnosis 32 months **after** PMF diagnosis	Possibly	Alive (72w)	Bonometti A, *Hum Pathol* 2018.
9	A	56y	M	L group	**Mixed LCH/ECD**	**ET**	Unknown (Mixed LCH/ECD) & NGS (ET)	***JAK2*** p.V617F	NR	Mixed LCH/ECD: ***BRAF*** p.V600E[6]; ET: ***TET2*** p.K95X[6]	N/A	N/A	Alive (N/A)	Papo M, Blood 2017.
10	A	N/A	N/A	L group	**Mixed LCH/ECD**	**AML-M4**	Sanger sequencing (LCH) & NGS (AML)	***TET2*** p.L1819X; ***SRSF2*** p.L95P	NR	Mixed LCH/ECD: ***BRAF*** p.V600E[6]	**Concurrent**	Yes	N/A	Durham BH, *Blood* 2017.
11	A	61y	M	L group	**ECD**	**AML-M5**[8]	NGS	***NRAS*** p.Q61R	NR	N/A	ECD diagnosis 27 months **before** AML diagnosis	Yes	Died (4m)	**This study**
12	A	80y	M	L group	**ECD**	**AML-M5**	Enhanced WES	***BRAF*** p.V600E, SI p.C635, *DNAH6* e61-23, *NDUFB4* NULL, *ENSG00000252849* NULL	NR	AML: *USP9X* p.K506fs, *LRRC31* e7+71, *MED12* p.G44D, *BICC1* p.R839C, *FAM47A* p.D275Y, *SPI1* p.A211fs; ECD: *PTPRN2* e10+6028	ECD diagnosis 2 years **before** AML diagnosis	Yes	Died (1m)	Ghobadi A, *Haematologica* 2019.
13	A	80y	M	L group	**ECD**	**AML NOS**[7]	At least BRAF-VE1	***BRAF*** p.V600E	NR	N/A	ECD diagnosis 2 years **before** AML diagnosis	N/A	N/A	Tzankov A, *Ann Hematol* 2018.
14	A	67y	M	L group	**ECD**	**CMML**	Unknown	***BRAF*** p.V600E, ***TET2*** and ***SRSF2*** mutations	NR	N/A	**Concurrent**	Yes	Alive (N/A)	Tzankov A, *Ann Hematol* 2018.
15	A	55y[9]	F	L group	**ECD**	**CMML**	NGS	***KRAS*** p.G12D, ***ASXL1*** p.G642fs	NR	N/A	ECD onset 9 years **before** CMML diagnosis	Yes	Alive (N/A)	Bonnet P, *Haematologica* 2019.
16	A	39y[9]	F	L group	**ECD**	**CMML**	NGS	***KRAS*** p.G12D, *DNMT3A* p.Y623fs	NR	N/A	ECD onset 9 years **before** CMML diagnosis	Yes	Alive (N/A)	Bonnet P, *Haematologica* 2019.
17	A	56y[9]	F	L group	**ECD**	**CMML**	WES	***KRAS*** p.G12D, ***ASXL1*** p.Y591X, *ROBO2* p.T982M, *CLDN1* p.A124T, *THBS4* p.R591W, *SYNC* p.E56K	NR	CMML: *RUNX* p.S501L; ECD: *AUTS2* p.H1133del, *OR10G3* p.G271V, *ROBO1* p.R1429H, *ZIC2* p.H239del, *ZNF774* p.G13R	ECD onset 3 years **before** CMML diagnosis	Yes	Died (2.5y)	Goyal G, *Br J Haematol* 2019.
18	A	54y[8]	F	L group	**ECD**	**CMML**	NGS	***NRAS*** p.G13D	NR	N/A	ECD onset 4 years **before** CMML diagnosis	Yes	Alive (N/A)	Bonnet P, *Haematologica* 2019.
19	A	66y	M	L group	**ECD**	**CMML**	NGS	***NRAS*** p.Q61R	NR	N/A	ECD diagnosis 2 years **before** CMML diagnosis	Yes	Alive (2m)	Papo M, *Blood* 2017.
20	A	66y	M	L group	**ECD**	**CMML**	Unknown	***NRAS*** p.Q61R	NR	N/A	ECD diagnosis 2 years **before** CMML diagnosis	N/A	Alive (1y)	Tzankov A, *Ann Hematol* 2018.
21	A	68y	M	L group	**ICH**	**CMML**	NGS	***NRAS*** p.G12V	NR	N/A	**Concurrent**	Yes	Died (2y)	**This study**
22	A	67y	M	L group	**ICH**	**CMML**	NGS	***KRAS*** p.G12R	NR	CMML: ***TET2*** p.M1333R and p.R1359Q[8]	ICH diagnosis 38 months **after** CMML diagnosis	Yes	Died (3m)	Loghavi S, *J Cutan Pathol* 2017.
23	A	81y[9]	M	L group	**ICH**	**CMML**	NGS (CMML) & multiplex PCR (ICH)	***TET2*** p.Q1466X and p.Q1523X, ***ASXL1*** p.K618X, *ZRSR2* p.Q100X	NR	N/A	ICH diagnosis **after** CMML diagnosis	Yes	Alive (2y)	Santos-Briz A, *Am J Dermatopathol* 2020.
24	P	3m	M	C group	**JXG**	**JMML**	NGS	***PTPN11*** p.E76K	NR	N/A	JXG diagnosis 23 months **before** JMML diagnosis	Possibly	Alive (18m)	Bátai B, *Pediatr Blood Cancer* 2020.
25	P	3y	F	L/M group	**Mixed LCH/LCS**	**T-ALL**	Sequencing of cloned PCR products	***NOTCH1*** p.C1693R and p.Q2441X	Yes (TCRγ)	N/A	LCH diagnosis 18 months **after** T-ALL diagnosis	No	Died (16m)	Rodig SJ, *Am J Hematol* 2008.
26	P	4y	M	UC/M group	**Mixed non-LCH/HS**	**T-ALL**	1. WES; 2. SNP microarray	1. *ACAC8* p.A507T, *ARHGEF11* UTR_C>T, *IGFN1* p.G12V, *MEIS1* p.G64G, *ODAM* p.R22H, *PPP5C* p.D453N; 2. Heterozygous CN loss at 9p21.3, including ***CDKN2A/B***	Yes (TCRβ and TCRγ)	T-ALL: A distinct biallelic loss at the ***CDKN2A/B*** locus; Non-LCH: A deletion of part of chromosome 8q (including ***MYC***); HS: Duplications of parts of chr. 5, chr. 11p, chr. 22q, and chr. 21q, as well as *C6orf211* p.R123I and *KIAA1644* p.R49W.	N/A and HS diagnosis 14 and 17 months **after** T-ALL diagnosis, respectively	Possibly	Died (3m)	Waanders E, *Leukemia* 2016.
27	A	47y	M	M group	**HS**	**CMML**	NGS	***KRAS*** p.A59E	NR	HS (presumed): ***MAP2K1*** p.F53L and ***RAF1*** p.S257L	**Concurrent**	Yes	Died (2m)	**This study**
28	N/A	N/A	N/A	M group	**HS**	**CMML**[10]	Pyrosequencing or NGS	A ***TP53*** mutation	NR	N/A	N/A	N/A	N/A	Facchetti F, *Virchows Arch* 2017.
29	A	26y	M	M group	**HS**	**MDS**[11]	NGS	***TP53*** and *BCOR* mutations	NR	HS: ***NRAS*** and *RARA* mutations[4]	HS diagnosis at least 1 year **after** MDS diagnosis	No	N/A	Tashkandi H, *Br J Haematol* 2020.
30	P	11y	N/A	M group	**HS**	**B/myeloid MPAL**	1. WES and low coverage WGS; 2. Methylation array	1. Mutations in *ADCY1* , *C9orf9* and *IGHV1-69* ; 2. CN loss at 14q, 12p and 9p, including homozygous deletion of ***CDKN2A*** , and gain of chr. X	N/A	MPAL: 1. 6 VUS, 2. CN loss at 7q, 9q, and 15q, and gain at 21q; HS: 1. ***KRAS*** p.A66fs and 4 VUS, 2. CN loss at 21q, and gain at 7p-q, 7q, 9q and 12p-q	HS diagnosis 5 months **after** B/myeloid MPAL diagnosis	Possibly	Died (N/A)	Bleeke M, *Pediatr Blood Cancer* 2020.
31	P	6y	M	M group	**HS**	**T-ALL**	Methylation array	CN loss at 14q and 9p, including ***CDKN2A***	Yes (TCR, not further specified)	N/A	HS diagnosis 16 months **after** T-ALL diagnosis	No	Died (1m)[12]	Bleeke M, *Pediatr Blood Cancer* 2020.
32	P	10y	M	M group	**HS**	**T-ALL**	Methylation array	CN loss at 9p, including ***CDKN2A***	Yes (TCR, not further specified)	T-ALL: CN loss at 14q; HS: CN loss at 8q and 16q	HS diagnosis 30 months **after** T-ALL diagnosis	No	Died (10m)	Bleeke M, *Pediatr Blood Cancer* 2020.
33	P	5m	M	M group	**HS**	**T-ALL**	Cytogenetic studies (T-ALL) & FISH (HS)	***MYC*** rearrangement	N/A	HS: ***BRAF*** p.V600E and ***CDKN2A*** copy loss[8]	HS diagnosis 14 months **after** T-ALL diagnosis	No	Alive (14m)[13]	Venkataraman V, *Pediatr Blood Cancer* 2020.
34	A	33y	M	M group	**HS**	**T-ALL**	1. FISH; 2. NGS	● ***MYC*** rearrangement; ● ***NRAS*** p.G12D, ***PTEN*** p.R234fs, ***PHF6*** p.R116X, ***NOTCH1*** p.R592H, *HOS1* p.R2072Q, *B2M* p.H71R, *LRP1B* p.L2794I	Yes (TCRαδ, TCRβ and TCRγ)	T-ALL: ***CDKN2A*** loss, *MTOR* p.T1977K and p.C1483Y, *PTCH1* p.I108M and *MYST3* p.R366Q; HS: ***RAF1*** p.G361A and p. V263A	HS diagnosis a few months **after** alloHSCT for T-ALL	No	Died (<1y)[14]	Roloff GW, *JCO Precision Oncology* 2019.
35	A	48y	M	M group	**HS**	**HCL**	1. BRAF pyrosequencing and BRAF-VE1; 2. aCGH	1. ***BRAF*** p.V600E; 2. CN losses at 7q, 10q, 11q, 17p (incl. ***TP53***) and 17q	Yes (Ig, not further specified)	HS: CN losses at 9p, 9q, 10p, 10q, 11p, 12p, 12q and 19p, and CN gains at 7p, 11p, 11q, 14q and 20q	HS diagnosis 26 years **after** HCL diagnosis	Yes	Died (9m)	Michonneau D, *J Clin Oncol* 2014.
36	A	N/A	N/A	M group	**HS**	**CLL**	WES	Mutations in ***BRAF*** and *ATM*; 12 VUS; del(11q)	Yes (IgHV)	HS: Mutations in ***NRAS*** and *NFB*, 13 VUS; del(4q) and del(8p)	HS diagnosis 7 years **after** CLL diagnosis	Yes[15]	Died (1w)[14]	Burger JA, *Nat Commun* 2016.
37	A	65y	M	M group	**HS**	**FL**	1. Karyotyping; 2. NGS (confirmed by HRM analysis and Sanger sequencing)	1. **t(14;18)**, gain of 9q and 19q; 2. ***CREBBP*** p.I1471T and p.F1484V, ***KMT2D*** p.D3061fs and p.L5318fs, *IGLL5* p.S58G and p.S61C, *TNFRSF14* p.W12X	Yes (IgH)	FL: 1. monosomy 5, loss of 6q, +mar(12); 2. *TNFAIP3* p.E374fs; HS: 1. gain of chromosomes 2, 3, and 8, loss of chromosome 12, and deletion of 13q; 2. ***KRAS*** p.G13D	**Concurrent**	Yes	Died (11m)	Péricart S, *Virchows Arch* 2019.
38	A	62y	M	M group	**HS**	**FL**	1. FISH; 2. PCR	**t(14;18)**; ***BCL2*** /*IH* MBR rearrangement	Yes (IgH)	N/A	HS diagnosis 2 years **after** first presentation with FL	No	N/A	Feldman AL, *Blood* 2008.
39	A	30y	F	M group	**HS**	**FL**	1. FISH; 2. PCR	**t(14;18)**; ***BCL2*** /*IH* MBR rearrangement	Yes (IgH)	N/A	HS diagnosis 12 years **after** first presentation with FL	No	N/A	Feldman AL, *Blood* 2008.
40	A	60y	M	M group	**HS**	**FL**[16]	1. FISH; 2. PCR	**t(14;18)**; ***BCL2*** /*IH* MBR rearrangement	No (clonal IgH rearrangement were detected in both neoplasms, but with unsatisfactory sequence similarity between the FL and HS)	N/A	HS diagnosis 3 years **after** first presentation with FL	No	N/A	Feldman AL, *Blood* 2008.
41	A	48y	F	M group	**HS**	**FL**	1. FISH; 2. PCR	**t(14;18)**; ***BCL2*** /*IH* MBR rearrangement	Yes (IgH)	N/A	HS diagnosis 2 months **after** first presentation with FL	N/A	N/A	Feldman AL, *Blood* 2008.
42	A	62y	M	M group	**HS**	**FL**	PCR	***BCL2*** /*IH* MBR rearrangement	No (clonal IgH rearrangement were detected in both neoplasms, but with unsatisfactory sequence similarity between the FL and HS)	N/A	**Concurrent**	Yes	N/A	Feldman AL, *Blood* 2008.
43	A	67y	M	M group	**HS**	**FL**	PCR	***BCL2*** /*IH* MBR rearrangement	No (no clonal IgH rearrangements in both neoplasms)	N/A	HS diagnosis 7 months **after** first presentation with FL	N/A	N/A	Feldman AL, *Blood* 2008.
44	A	75y	F	M group	**HS**	**FL**	1. FISH; 2. PCR & sequencing	**t(14;18)**; fused *IGH–BCL2* amplicons	Yes (IgH)	FL: multiple CN aberrations	HS diagnosis 6 years **after** FL diagnosis	Possibly	Died (2m)	Brunner P, *Leukemia* 2014.
45	A	39y	M	M group	**HS**	**FL**	1. FISH; 2. PCR	**t(14;18)**; *IGH*/***BCL2*** MBR rearrangement	Yes (IgH)	N/A	HS diagnosis 4 years **after** FL diagnosis	No	Died (9m)	Zeng W, *J Cutan Pathol* 2011.
46	N/A	N/A	F	M group	**LCS**	**FL**[17]	1. FISH; 2. WES; 3. SNP microarray	1. **t(14;18)** & ***MYC*** extra copies; 2. ***CREBBP*** p.1640_1641del[18]; 3. CN gain at 22q11.23	No (FL was positive for clonal IgH gene rearrangements, but the LCS sample was negative for clonal IgH gene rearrangements)	FL: 2. ***CDKN2A*** p.M1T, ***KMT2D*** p.R5097X, ***BCL2*** p.S203N and p.A45T, *SMARCA4* p.G883D, *IL2* p.V89L, *MLLT3* deletion, *MN1* p.524_524del and p.304_304del; 3. multiple CN aberrations; LCS: 2. ***KRAS*** p.G13D, ***NOTCH1*** p.2411_2411del, *BIN1* p.H283Y, *TMEM200A* p.G383R, *DNAJB12* p.R13W, *TRPV42* p.R249H, *ADAD2* p.R299Q, *KRTAP4–7* p.S113C, *EMR2* p.C520F, *ARMCX4* p.G1814E, p.E1816G and p.G1820E, *CAMTA1* p.N1271fs, *BCR* p.G1049fs, *ARID1A* p.1333_1334del	LCS diagnosis 10 years **after** FL diagnosis	No	Died (<1y)	Choi SM, *Diagn Pathol* 2018.
47	A	77y	M	M group	**LCS**	**MZL**	aCGH	Trisomy 12; del(11q14.1q24.1)	Yes (IgHV and IgH)	LCS: dup(16q21q23.1) and dup(17q12q22)	LCS diagnosis 6 years **after** MZL diagnosis	Yes	Died (2m)	Ambrosio MR, *Virchows Arch* 2015.
48	A	55y	M	M group	**LCS**	**CLL**	1. NGS (confirmed by Sanger sequencing); 2. aCGH	1. ***NRAS*** p.Q61K, ***NOTCH1*** p.Q2403X, ***KMT2D*** p.E2989X, ***MAP2K1*** p.C121S; 2. CN loss at 9p21, including ***CDKN2A/B***	Yes (IgH)	N/A	LCS diagnosis 4 years **before** CLL diagnosis	Yes	Died (few months)	Facchetti F, *Virchows Arch* 2017. & Xerri L, *Am J Surg Pathol* 2018.
49	A	74y	M	M group	**IDCS**	**CLL/SLL**	FISH & aCGH	Trisomy 12	Yes (IgH)	IDCS: chromosomal gains in part of chr. 16q	IDCS diagnosis 3 years **after** CLL/SLL diagnosis	Yes	N/A	Fraser CR, *Am J Clin Pathol* 2009.
50	A	52y	M	M group	**IDCS**	**DLBCL**	1. FISH; 2. WES	1. ***MYC*** translocation; 2. ***MYC*** 29 mutations in exon 2, ***TP53*** p.S94X, ***PTEN*** splicing mutation, *BAIAP3* p.D780Y, *PRPH2* p.N199S, *PCDHB1* p.S243L; CN gain at 6p, 10p and 16p, and CN loss at chr18, and uniparental disomy of 10q (including ***PTEN***)	Yes (IgH)	DLBCL: CN loss at 15q and 19q, and CN gain at 4q and chr12; IDCS: ***KMT2D*** p.V5180G, *ERBB3* p.T1028K, *SETD2* p.Q2284K; CN loss at 4p, 4q, 9p and chr22, and gain at 5p, 5q, 7p and 8q.	IDCS diagnosis 15 months **after** DLBCL diagnosis	No	N/A	Ochi Y, *Haematologica* 2018.
51	A	86y	F	UC	**Atyp. non-LCH**	**PTCL NOS**	NGS (confirmed by pyrosequencing)	***NRAS*** p.Q61K	Yes (TCRβ and TCRγ; IgH)	N/A	Atypical non-LCH diagnosis 3.5 years **before** PTCL diagnosis	No	Died (<1y)	Machan S, *Am J Dermatopathol* 2019.
52	A	23y	F	UC	**Atyp. non-LCH**	**AML-M0**	Sanger sequencing (non-LCH) & NGS (AML)	***RUNX1*** p.R166X and p.P425L	NR	N/A[19]	Non-LCH diagnosis 6 months **before** AML diagnosis[20]	No[21]	Died (<1y)	Al Mugairi A, *Am J Clin Pathol* 2019.
53	A	65y	M	NC	**MPDCN**	**MDS-MLD**	NGS	***PTPN11*** p.R501K	NR	MDS: del(6)(p25.2), del(6)(p23p22.3), del(17)(q11.2) and a CN-LOH in 21q	Mature PDCN diagnosis 1 year **after** MDS diagnosis	Yes	Died (1y)	Bodmer A, *Virchows Arch* 2017.
54	A	78y	M	NC	**BPDCN**	**AML NOS**	NGS	***TET2*** p.C1642fs and p.A1810fs, ***SRSF2*** p.P95H	NR	BPDCN: CN loss at 3p, 5q, 7p, 9p, 12p, 13q, Xp (incl. ***CDKN2A*** , ***RB1*** & *IKZF1*) AML: ***CREBBP*** p.Y1733fs, ***RUNX1*** p.F116S, ***ASXL1*** p.E635fs, *CSF3R* p.T618I, ***NRAS*** p.G612D and p.G12V and p.G12S, and *FLT3* -ITD.	BPDCN diagnosis 14 months **after** AML diagnosis	Yes	Died (<1y)	Luskin MR, *Leuk Lymphoma* 2020.
55	A	61y	M	NC	**BPDCN**	**CMML**	WES	***TET2*** p.G523fs, ***SRSF2*** p.P95L, ***PHF6*** p.Q251H, *PLCXD3* p.T282M, *TRMT61B* p.Y219C[22], *STK3* p.R178Ts[22], *SLC25A10* p.R263C, *DIP2A* p.R309W[22], *SARDH* p.G641E, *PLP1* p.T116M	NR	CMML: *IVL* 227_236del; BPDCN: ***RB1*** p.R251X (and LOH of 13.q13-24, involving the ***RB1*** gene locus), *CROCC* p.T1532S, *ERCC4* p.V870I, *CHP2* p.R34Q	BPDCN diagnosis 3 years **after** CMML diagnosis[23]	No[24]	Died (<1y)	Patnaik MM, *Blood Cancer J* 2018.
56	A	80y	M	NC	**BPDCN**	**CMML**	NGS	***TET2*** p.Y1244fs and p.Q810X, ***SRSF2*** p.P95H	NR	CMML: ***JAK2*** p.V617F	BPDCN diagnosis 2 years **after** CMML diagnosis	Yes	Died (<1yr)	Brunetti L, *Leukemia* 2017.
57	N/A	N/A	N/A	NC	**BPDCN**	**CMML**	Unknown	A ***TET2*** mutation	NR	CMML: another ***TET2*** mutation; BPDCN: another ***TET2*** mutation	BPDCN diagnosis **before** CMML diagnosis	Yes	N/A	Sukegawa S, *Rinsho Ketsueki* 2018.
58	A	53y	M	NC	**BPDCN**	**MDS-RARS**	Unknown	A ***TET2*** mutation	NR		BPDCN diagnosis 3 years **after** MDS-RARS diagnosis	No[24]	Alive (<1y)	Krause JR, *Proc (Bayl Univ Med Cent)* 2017.

Extra abbreviations: UC, unclassifiable; NC, not classified; WES, whole-exome sequencing; SNP, single-nucleotide polymorphism; PCR, polymerase chain reaction; NGS, next-generation sequencing; FISH, fluorescence in situ hybridisation; WGS, whole-genome sequencing; aCGH, array comparative genomic hybridisation; HRM, high resolution melt analysis; NR, not relevant.

[1] According to the revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages (Emile JF, et al. *Blood* 2016).

[2] Follow-up is depicted as the time in months (m) or years (y) since the diagnosis of the last presenting disorder (histiocytic neoplasm or additional haematological malignancy).

[3] A *NRAS* p.G12S mutation and homozygous deletion at 9p21, including *CDKN2A*, was also detected in the T-ALL sample using WES and SNP array analysis. LCH specimens were not available for these analyses.

[4] LCH was first diagnosed in the temporal bone 40 years before HL diagnosis. One year after complete remission for HL, LCH was diagnosed for the second time in the os ileum. The molecular analysis was performed on the os ileum LCH sample.

[5] This patient developed evolving acute myeloid leukaemia in the setting of chronic high-grade myelodysplastic syndrome and refractory anaemia with excess blasts. Targeted treatment with enasidenib, an *IDH2* inhibitor, for progression of the myelodysplastic syndrome resulted in stable cutaneous LCH. Thus, at least the patient's LCH and MDS were concurrently present.

[6] The patient was initially diagnosed with concurrent ECD and multiple myeloma. Molecular analysis of the multiple myeloma was precluded by the absence of material.

[7] AML with at least phenotypic monocytic differentiation.

[8] Age at onset of xanthelasma-like ECD lesions, not age at diagnosis of ECD.

[9] Age at onset of the ICH lesion, not age at onset of the first presenting disorder (CMML), as this age was not reported.

[10] The patient also had a mediastinal germ cell tumour (MGCT). The existence of a common precursor was suggested by the demonstration of a *TP53* mutation in all three neoplasms (MGCT, HS, CMML) and identical chromosomal aberrations in the HS and the MGCT.

[11] The patient also had a metastatic non-seminomatous germ cell tumour with yolk sac component (NSGCT). The existence of a common precursor was suggested by the demonstration of the same *TP53* and *BCOR* mutations in all three neoplasms (NSGCT, HS, MDS).

[12] Died after this time period after the onset of the HS. The diagnosis of HS was made post-mortem.

[13] The histiocytic sarcoma responded to targeted therapy with dabrafenib and trametinib.

[14] Off-label trametinib was obtained, and the patient started therapy as a maintenance approach. Unfortunately, symptoms of graft-versus-host disease worsened, and treatment had to be discontinued after 2 weeks.

[15] Although histopathological examination of bone marrow revealed only 2% involvement by CLL cells (compared with 40% before starting ibrutinib therapy).

[16] The original FL was accompanied by an unusual histiocytic reaction without overt malignant features.

[17] The patient also developed DLBCL 7 years before and several months after LCS diagnosis. Molecular analysis of these tumours was precluded by the absence of material.

[18] In addition, 11 variants that are presumably germline polymorphisms were detected.

[19] *FLT3* p.A680V, *KRAS* p.V14I, *ETV6* p.R378X, *EZH2* p.V626M, *GATA2* loss, *BCOR* p.R342X, *PTPN11* p.T553M, and *PAK3* p.S156L were detected using NGS in the AML sample. The non-LCH samples were not analyzed for genetic alterations involving these genes.

[20] At diagnosis of non-LCH, complete blood count already revealed 2% blasts. Thus, there may have been concurrent AML at non-LCH diagnosis.

[21] Non-LCH evolved to AML in bone marrow.

[22] These mutations are depicted as such in Figure 2A of the manuscript (Patnaik MM, *Blood Cancer J* 2018), but other mutations in *TRMT61B* (p.T219C), *STK3* (p.N207fs) and *DIP2A* (p.R373W) are described in the text of this article.

[23] At diagnosis of CMML, plasmacytoid dendritic cell nodules were present in a bone marrow biopsy, comprising ~20% of bone marrow cellularity.

[24] The additional haematological malignancy evolved to BPDCN in the bone marrow.

[#] On condition that the histiocytic neoplasm and/or additional haematological malignancy was/were analysed for the genetic alteration(s) detected in the associated neoplasm. This information was not reported.

Table S3. Reported patients with histiocytic neoplasms associated with additional haematological malignancies bearing the same genetic alteration(s) as demonstrated by techniques other than DNA sequencing and/or DNA methylation profiling.

Nr	P/A	Age	G	Histiocytic classification[1]	Histiocytic neoplasm	Additional haematological malignancy	Analysis method	Shared genetic alteration(s)	Shared *TCR* or Ig rearrangements	Unique genetic alteration(s)	Interval	Concurrently present	Outcome (follow-up)[2]	Reference
1	A	61y	F	L group	**LCH**	**MS/AML-M4**	Cytogenetics (AML) and FISH (LCH, MS and AML)	Trisomy 8	NR	N/A	**Concurrent**[3]	Yes	Died (4m)	Schmitt-Graeff AH, *Leukemia* 2012.
2	A	68y	M	L group	**LCH**	**T/myeloid MPAL**	FISH	Trisomy 21	N/A	N/A	**Concurrent**	Yes	N/A	Yohe SL, *Mod Pathol* 2014.
3	A	52y	F	L group	**LCH**	**CLL**	FISH	Del(17p)	Yes (IgH)	LCH: ***TP53*** p.P92L, ***BRAF*** p.V600E and *STK11* p.M335T[#]; CLL: ***TP53*** p.P191del	LCH diagnosis 11 years **after** CLL diagnosis	Yes[4]	N/A	Frauenfeld L, *Virchows Arch* 2019.
4	N/A	N/A	N/A	L group	**LCH**	**FL**	N/A	**t(14;18)** *IGH*- ***BCL2*** fusion	N/A	N/A[5]	LCH diagnosis 5 years **after** FL diagnosis	N/A	N/A	Facchetti F, *Virchows Archiv* 2017.
5	A	77y	F	L group	**LCH**	**FL**	FISH	**t(14;18)** *IGH*– ***BCL2*** fusion	Yes (IgK)	N/A	**Concurrent**	Yes	N/A	West DS, *Am J Surg Pathol* 2013.
6	A	59y[6]	M	C group	**RH**	**SM & AML**	FISH (RH); karyotyping (mixed RH, SM & AML)[7]	Der(1;9)	NR	N/A	RH diagnosis 1 year **before** SM & AML diagnosis	Yes	Alive (30m)	Fusco N, *Histopathology* 2017.
7	A	84y	M	C group	**GEH**	**CMML**	FISH (GEH); karyotyping (CMML)	Loss of Y chromosome	NR	N/A[8]	GEH diagnosis shortly **after** CMML diagnosis	Yes	Died (4m)	Shon W, *J Cutan Pathol* 2013.
8	A	70y	M	M group	**HS**	**CMML**	FISH	Aneuploidy of chromosome 8	NR	HS cells were tetraploid, while CMML cells were diploid.	**Concurrent**	Yes	Alive (<1y)	Mori M, *Int J Hematol* 2010.
9	A	72y[9]	M	M group	**HS**	**CML**	FISH (HS); N/A (CML)	t(9;22) *BCR-ABL1* fusion	NR	N/A[10]	HS diagnosis 30 months **after** CML diagnosis	No	Died (1m)	Ansari J, *Eur J Haematol* 2016.
10	P	5y	M	M group	**HS**	**T-ALL**	FISH (HS); karyotyping (T-ALL)	t(7,11) ***CDKN2A*** deletion on both chromosomes 9	N/A	N/A	HS diagnosis 6 months **after** T-ALL diagnosis	No	Died (N/A)	Castro ECC, *Pediatr Dev Pathol* 2010.
11	P	4y	M	M group	**HS**	**B-ALL**	FISH (non-LCH & B-ALL); karyotyping (B-ALL)	Homozygous deletion of the ***CDKN2A*** locus at 9p21	Yes (TCRγ and IgH)	N/A	HS diagnosis several months **after** B-ALL diagnosis	No	Died (1y)	Kumar R, *Pediatr Blood Cancer* 2011.
12	P	7y	M	M group	**HS**	**B-ALL**	FISH (HS); karyotyping (B-ALL)	**t(8;14)**, including ***MYC***	N/A	N/A	HS diagnosis 6 months **after** B-ALL diagnosis	No	Alive (N/A)	Castro ECC, *Pediatr Dev Pathol* 2010.
13	A	64y	F	M group	**HS**	**CLL**	Karyotyping	Trisomy 12	N/A for the CLL	HS: (t5;14)(q32;q32), +der(12), t(8;12)(qter > q21;p21 > q22), +der(21), t(21;?)(qter;?)	HS diagnosis 8 years **after** CLL diagnosis	Yes	Died (1.5m)	Wetzler M, *Cancer* 1995.
14	A	70y	M	M group	**HS**	**CLL/SLL**	FISH	Homozygous deletion of 13q14	N/A	HS: a complex, near-tetraploid karyotype	HS diagnosis 8 years **after** CLL diagnosis	Yes	Died (3m)	Skala SL, *Clin Pathol* 2019.
15	A	85y	M	M group	**HS**	**CLL/SLL**	FISH	Del(17p)	Yes (IgH)	IDCS: deletion of 13q	**Concurrent**	Yes	N/A	Shao H, *Mod Pathol* 2011.
16	N/A	N/A	N/A	M group	**HS**	**MM**	N/A	t(11;14) ***CCND1***-*IgH* fusion	Yes (IgH and IgK)	N/A	HS diagnosis 1 year **after** MM diagnosis	N/A	N/A[11]	Facchetti F, *Virchows Archiv* 2017.
17	A	56y	F	M group	**HS**	**MCL**	FISH	t(11;14) ***CCND1***-*IgH* fusion	Yes (IgH)	N/A	HS diagnosis >2.5 years **after** MCL diagnosis	Yes	Alive (N/A)	Hure MC, *J Clin Oncol* 2012.
18	A	61y	F	M group	**HS**	**DLBCL**[12]	FISH	**t(14;18)** *IGH*– ***BCL2*** fusion	Yes (IgH)	N/A	HS diagnosis 17 months **before** DLBCL diagnosis	No[13]	Died (2.5m)	Wang E, *Am J Surg Pathol* 2011.
19	A	50y	M	M group	**HS**	**DLBCL & FL**	FISH	**t(14;18)** *IGH*– ***BCL2*** fusion	N/A	N/A	**Concurrent**	Yes	N/A	Zhang D, *Int J Hematol* 2009.
20	A	53y	M	M group	**HS**	**DLBCL & FL**	FISH & karyotyping	**t(14;18)**(18q21)	Yes (IgH, between the HS and FL; DLBCL not tested)	HS: +X, add(1)(p36),add(6)(q27), del(6)(q23),del(9)(p22), +12,dup(13)(q21q31),[cp5]; FL: +2,del(6)(q14), +8,t(14;18)(q32;q21), +17[cp8].	Concurrent HS and DLBCL diagnosed 13 years **after** FL diagnosis	Yes	Died (<1y)	Bassarova A, *J Hematop* 2009.
21	P	1y	M	M group	**HS**	**BL**	N/A	**t(8;14)** *IGH*-***MYC*** fusion	N/A	N/A	N/A	N/A	Died (N/A)	Minard-Colin V, *NEJM* 2020.
22	A	76y[14]	F	M group	**HS**	**FL**	N/A	***BCL2*** / ***MYC*** double hit translocation	Yes (IgH)	N/A	HS diagnosis 8 years **after** FL diagnosis	Yes	N/A	Facchetti F, *Virchows Archiv* 2017.
23	N/A	N/A	N/A	M group	**HS**	**FL**	N/A	***BCL2*** rearrangement	N/A	N/A[15]	HS diagnosis 1 year **after** FL diagnosis	N/A	Alive (N/A)[16]	Facchetti F, *Virchows Archiv* 2017.
24	A	77y	F	M group	**HS**	**FL**	FISH[17]	***BCL2*** translocation	N/A	N/A	**Concurrent**	Yes	N/A	Fernandez-Pol S, *Hum Pathol* 2016.
25	A	62y	F	M group	**HS**	**FL**	FISH	**t(14;18)** *IGH*– ***BCL2*** fusion	N/A	N/A[18]	HS diagnosis 2 years **after** FL diagnosis	Yes	N/A	Mehrotra S, *Diagn Cytopathol* 2015.
26	A	75y[19]	M	M group	**HS**	**FL**	FISH	***BCL2*** rearrangement	N/A	N/A	HS diagnosis 13 years **after** FL diagnosis	Yes	Alive (16m)	Farris M, *Clin Lymphoma Myeloma Leuk* 2019.
27	N/A	N/A	N/A	M group	**LCS**	**FL**	N/A	**t(14;18)** *IGH*- ***BCL2*** fusion	No (only IGH clonality in the FL)	N/A	LCS diagnosis 10 years **after** FL diagnosis	No	N/A	Facchetti F, *Virchows Archiv* 2017.
28	A	66y	M	M group	**LCS**	**FL**	FISH	*BCL6* split	Yes (IgH)	N/A	**Concurrent**	Yes	Died (<1m)	Shimono J, *Pathol Int* 2018.
29	A	62y	M	M group	**LCS**	**DLBCL**	FISH	**t(14;18)**(18q21)	Yes (IgH)	N/A	HS diagnosis 1 year **after** DLBCL diagnosis	No	Died (<1y)	Bassarova A, et al. *J Hematop* 2009.
30	A	68y[20]	F	M group	**LCS**	**CLL/SLL**	FISH	Loss of 6q23	N/A	LCS: ***BRAF*** p.V600E	LCS diagnosis 6 years **after** CLL diagnosis	Yes	Died (<1y)	Chen W, *N Am J Med Sci* 2013.
31	A	66y	F	M group	**LCS**	**HCL**	Karyotyping	+4,del(6)(q23),del(8)(p21)x2,+12, del(14)(q24), add(17)(p13)	Yes (Ig, not further specified)	LCS: monosomy 13	LCS diagnosis 8.5 years **after** HCL diagnosis	Yes	Died (<1y)	Muslimani A, *Ann Hematol* 2012.
32	N/A	N/A	N/A	M group	**LCS**	**T-ALL**	N/A	Deletions of *TLX3* at 5q35, *TRA/TRD* at 14q11 and *TRG* at 7p14	Yes (TCRβ and TCRγ)	N/A[21]	LCS diagnosis 2 years **after** T-ALL diagnosis	No	N/A	Facchetti F, *Virchows Archiv* 2017.
33	A	59y	F	M group	**ICS**	**T-LBL**	FISH	Trisomy 21	N/A	T-LBL: ***NRAS*** p.G13D and monosomy 18	**Concurrent**	Yes	Died (3y)	Buser L, *Pathobiology* 2014.
34	A	65y[22]	M	M group	**IDCS**	**CLL/SLL**	FISH	Del(17p)	Yes (IgK)	N/A	IDCS diagnosis 3 years **after** CLL/SLL diagnosis	Yes	Died (4.5m)	Shao H, *Mod Pathol* 2011.
35	A	55y	F	M group	**IDCS**	**FL**	FISH	**t(14;18)**	Yes (IgH)	N/A	**Concurrent**	Yes	N/A	Feldman AL, *Blood* 2008.
36	A	66y	M	UC	**Atyp. non-LCH**	**B-ALL**	FISH (non-LCH & B-ALL); karyotyping (B-ALL)	Trisomy 11	N/A	N/A	Atyp. non-LCH diagnosis 18 months **after** B-ALL diagnosis	No	Alive (N/A)	Castro ECC, *Pediatr Dev Pathol* 2010.
37	A	18y	M	UC	**Atyp. non-LCH**	**B-ALL**	FISH (non-LCH & B-ALL); karyotyping (B-ALL)	Trisomy 5	N/A	N/A	Atyp. non-LCH diagnosis 16 months **after** B-ALL diagnosis	No	Died (N/A)	Castro ECC, *Pediatr Dev Pathol* 2010.
38	A	59y	F	NC	**BIDCT**	**CMML**	FISH	Trisomy 8	N/A	N/A	BIDCT diagnosis **after** CMML diagnosis	N/A	Alive (4.5m)	Vitte F, *Am J Surg Pathol* 2012.

Extra abbreviations: RH, reticulohistiocytosis; GEH, generalised eruptive histiocytosis; ICS, indeterminate cell sarcoma; BIDCT, blastic indeterminate dendritic cell tumour; MS, myeloid sarcoma; SM, systemic mastocytosis; CML, chronic myeloid leukaemia; MM, multiple myeloma; MCL, mantle cell lymphoma; BL, Burkitt's lymphoma; LBL, lymphoblastic lymphoma.
[1] According to the revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages (Emile JF, et al. *Blood* 2016).
[2] Follow-up is depicted as the time in months (m) or years (y) since the diagnosis of the last presenting disorder (histiocytic neoplasm or additional haematological malignancy).
[3] A bone marrow aspirate at diagnosis of mixed LCH/MS revealed 7% myeloblasts and an isolated trisomy 8, consistent with a refractory anaemia with blast excess-1 (RAEB-1). One month after mixed LCH/MS diagnosis, overt AML developed.
[4] In the LCH sample, the *TP53* p.P191del mutation was detected with a VAF of 3%, probably due to rare contaminating CLL cells.
[5] The LCH sample stained positive for BRAF-VE1. It was not reported whether a FL specimen was stained for BRAF-VE1.
[6] The patient had been diagnosed with RH 1 year earlier.
[7] Karyotyping was performed on whole bone marrow, consisting of SM, AML, and a nodular histiocytic infiltrate consistent with localisation of reticulohistiocytosis. Therefore, the authors could not formally demonstrate the cellular origin of the clones bearing the cytogenetic alterations that were detected in the bone marrow, and which were confirmed in the RH-affected dermal lesions. Thus, it is not completely certain that the SM & AML carried the der(1;9).
[8] A *JAK2* p.V617F mutation was detected in the CMML. It was not reported whether the GEH was analysed for this mutation as well.
[9] The patient was diagnosed with CML 30 months prior.
[10] WES of the HS revealed 11,893 missense single nucleotide variants (SNVs), 192 insertion variants, and 292 deletion variants (indels) which cause frameshifts. Among common B-cell-regulating genes, missense variants were identified in two genes, including *EBF1* (p.R381G) and *BLIMP1* (p.D203E). Heterozygous SNV was also seen in *BRAF* (rs140449071, rs140449150).
[11] The patient was unsuccesfully treated with Velcade + Dexamethasone.
[12] This patient had a remote history of FL, diagnosed 17 years before HS diagnosis. On account of the remote clinical history, the original specimen with FL could not be obtained, precluding genotypic comparison of IgH gene rearrangement products between the original FL and subsequent HS or DLBCL by the authors.
[13] At time of HS diagnosis, a staging bone marrow biopsy showed a low level of involvement by FL, but no evidence of HS. Thus, FL and HS were concurrently present.
[14] Age at diagnosis of the follicular lymphoma.
[15] The HS stained positive for BRAF-VE1. In addition, mutations involving the genes *BCL2*, *BCL10*, *CDKN1B*, *KIT* and *MAP2K1* were detected. It was not reported whether the FL was analysed for these genetic alterations as well.
[16] The patient was unsuccessfully treated with Rituximab + Bendamustine. Yet, a MEK inhibitor as single agent induced a complete response in this highly compromised patient with multifocal disease.
[17] Apart from the HS-affected liver biopsy, FISH was performed on a mixed HS/FL bone marrow biopsy, revealing "a variable 5'/3' *BCL2* signal separation pattern in 18 (9%) of nuclei, further supporting the diagnosis of follicular lymphoma". The authors did not report the type of cells that showed the *BCL2* rearrangement.
[18] A *BRAF* p. V600E mutation was detected in the HS. It was not reported whether the FL was analysed for this mutation as well.
[19] Age at diagnosis of mixed HS/FL. The patient was diagnosed with FL 13 years before.
[20] Six years before, the patient was diagnosed with CLL.
[21] A *BRAF* p.V600E mutation was detected in the LCS using BRAF-VE1 immunohistochemistry and direct sequencing. It was not reported whether the T-ALL was analysed for this mutation as well.
[22] Age at presentation with the IDCS.
[#] On condition that the histiocytic neoplasm and/or additional haematological malignancy was/were analysed for the genetic alteration(s) detected in the associated neoplasm. This information was not reported.

Case 1

First bone marrow biopsy (HS)

Next generation sequencing

Material: hematoxylin-stained slides
NGTS panel: Cancer Hotspot Panel version 2.0 (CHPv2.0)
NGTS platform: Single molecule Molecular Inversion Probe (smMIP)-based sequence analysis using a NextSeq 500 sequencer.
Genes analyzed: AKT1 [NM_005163.2]: codon 17, BRAF [NM_004333.4]: codon 582-615, CTNNB1 [NM_001904.3]: codon 19-48, CXCR4 [NM-001008540.1]: codon 281-357, EGFR [NM_005228.3]: codon 434-499, 688-823, 849-875, ERBB2 [NM_004448.3]: codon 770-785, EZH2 [NM_004456.4]: codon 471-502, 618-645, 679-704, GNA11 [NM_002067.4]: codon 183 and 209, GNAQ [NM_002072.4]: codon 183 and 209 , GNAS [NM_000516.5]: codon 201 and 227 , H3F3A [NM_002107.4]: codon 28 and 35, H3F3B [NM_005324.4]: codon 37, HRAS [NM_005343.3]: codon 12, 13, 59 and 61, IDH1 [NM_005896.3]: codon 132, IDH2 [NM_002168.3]: codon 140 and 172, JAK2 [NM_004972.3]: codon 617, KIT [NM_000222.2]: codon 412-513, 550-591, 628-713, 799-828, KRAS [NM_004985.4]: codon 12, 13, 59, 61, 117 and 146, MPL [NM_005373.2]: codon 515, MYD88 [NM_002468.4]: codon 169-280, NRAS [NM_002524.4]: codon 12, 13, 59, 61, 117 and 146, PDGFRA [NM_006206.4]: codon 552-596, 632-667, 814-848, PIK3CA [NM_006218.2]: codon 520-554, 1020-1069, SF3B1 [NM_012433.2]: codon 603-671, 694-727, 833-906.

Variant(s) detected:
Mutation in KRAS [NM_004985.4] exon 3; c.176C>A; p.A59E, variant allele frequency: 30%, 65/217 unique reads.

Next generation sequencing

Material: hematoxylin-stained slides
NGTS panel: Predictive Analysis for THerapy version 2.0 DNA (PATHv2D) Panel
NGTS platform: Single molecule Molecular Inversion Probe (smMIP)-based sequence analysis using a NextSeq 500 sequencer.
Genes analyzed: AKT1 [NM_005163.2]: codon 17, AKT2 [NM_001626.5]: codon 17 , AKT3 [NM_181690.2]: codon 17 , ALK [NM_004304.4]: codon 1059-1150, 1173-1278, ARAF [NM_001654.4]: codon 214 , BRAF [NM_004333.4]: codon 455-488, 566-580, 594-605, DDR2 [NM_006182.2]: codon 503-856, EGFR [NM_005228.4]: codon 434-499, 688-875, ERBB2 [NM_004448.3]: codon 310, 650-695, 737-883, GNA11 [NM_002067.4]: codon 183 and 209, GNAQ [NM_002072.4]: codon 183 and 209 , GNAS [NM_000516.5]: codon 201 and 227 , HRAS [NM_005343.3]: codon 12, 13, 59 and 61, IDH1 [NM_005896.3]: codon 132, IDH2 [NM_002168.3]: codon 140 and 172, JAK2 [NM_004972.3]: codon 617, KIT [NM_000222.2]: codon 412-513, 550-591, 640-787, 799-850, KRAS [NM_004985.4]: codon 12, 13, 59, 61, 117 and 146, MAP2K1 [NM_002755.3]: codon 28-231, MET [NM_001127500.2]: codon 168, 375, 982-1027, 1230-1284, 1304, including exon 14 (-90, +20bp), MTOR [NM_004958.3]: 1458-1489, 1789-1820, 1971-1995, 2194-2220, 2404-2433, 2484-2509, NRAS [NM_002524.4]: codon 12, 13, 59, 61, 117 and 146, PDGFRA [NM_006206.5]: codon 552-595, 632-667, 824-848, PIK3CA [NM_006218.3]: codon 345, 420, 539-554, 1043-1050, POLE [NM_006231.3]: codon 268-491, PTEN [NM_000314.6]: codon 86-267, 276-342, RAF1 [NM_002880.3]: codon 257-261, ROS1 [NM_002944.2]: codon 1927-2189, TP53 [NM_000546.5]: >94% of the coding sequence, markers BAT25, BAT26, NR21, NR24 and NR27 for microsatellite instability (MSI) analysis. Gene amplifications: ALK, BRAF, EGFR, ERBB2, FGFR1 [NM_001174063.1], FGFR2 [NM_000141.4], FGFR3 [NM_000142.4], KIT, KRAS, MDM2 [NM_002392.5], MET, PDGFRA, PIK3CA.

Variant(s) detected:
Mutation in KRAS [NM_004985.4] exon 3; c.176C>A; p.A59E, variant allele frequency: 34%, 55/162 unique reads.
Mutation in MAP2K1 [NM_002755.3] exon 2; c.159T>G; p.F53L, variant allele frequency: 3.8%, 15/395 unique reads.
Mutation in RAF1 [NM_002880.3] exon 7; c.770C>T; p.S257L, variant allele frequency: 7.9%, 31/392 unique reads.

Second bone marrow biopsy (CMML/HS)

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: CHPv2.0
NGTS platform: smMIP-based sequence analysis using a NextSeq 500 sequencer.

Variant(s) detected:
Mutation in KRAS [NM_004985.4] exon 3; c.176C>A; p.A59E, variant allele frequency: 40%, 30/75 unique reads.

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: PATHv2D Panel
NGTS platform: smMIP-based sequence analysis using a NextSeq 500 sequencer.

Variant(s) detected:
Mutation in KRAS [NM_004985.4] exon 3; c.176C>A; p.A59E, variant allele frequency: 42%, 50/120 unique reads.
Mutation in MAP2K1 [NM_002755.3] exon 2; c.159T>G; p.F53L, variant allele frequency: 2%, 8/404 unique reads.

Variant with too low coverage for adequate variant calling:
Mutation in RAF1 [NM_002880.3] exon 7; c.770C>T; p.S257L, variant allele frequency: 0.7%, 2/306 unique reads.
NOTE: This is below the detection limit of the NGS assay. In addition, it comprises a C>T change, and may be an FFPE artefact.

Case 2

Skin biopsy (ICH)

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: NGS OPv3.0
Genes (exons) analyzed:
AKT1 [NM_005163] (3, 6), ALK [NM_004304] (15, 22-25), BRAF [NM_004333] (11, 15), CALR [NM_004343] (9), CD79B [NM_001039933] (5), CTNNB1 [NM_001904] (3), EGFR [NM_005228] (12, 18-21), ERBB2 [NM_004448] (8, 19-21), FGFR1 [NM_023110] (4, 7), FGFR2 [NM_001144915] (6, 8, 11), FGFR3 [NM_001163213] (7, 9, 14, 16, 18), FOXL2 [NM_023067] (1), GNA11 [NM_002067] (4, 5), GNAQ [NM_002072] (4, 5), GNAS [NM_000516] (8, 9), H3F3A [NM_002107] (2), H3F3B [NM_005324] (2), HRAS [NM_176795] (2,3), IDH1 [NM_005896] (4), IDH2 [NM_002168] (4), JAK2 [NM_004972] (14), KIT [NM_000222] (2, 8-11, 13-15, 17, 18), KRAS [NM_033360] (2-4), MAP2K1 [NM_002755] (2, 3, 6), MET [NM_000245] (2, 14, 16, 19), MPL [NM_005373] (10), MYD88 [NM_002468] (3, 5), NOTCH1 [NM_017617] (26, 27, 34), NRAS [NM_002524] (2-4), PDGFRA [NM_006206] (12, 14, 15, 18), PIK3CA [NM_006218] (10, 14, 21), RB1 [NM_000321] (4, 6, 10, 11, 14, 17, 18, 20-22), RET [NM_020975] (10, 11, 13, 15, 16), RHOA [NM_001664] (2), SMAD4 [NM_005359] (3-6, 8-12), TP53 [NM_000546] (2, 4-8, 10).

Variant(s) detected:
Mutation in NRAS (NM_002524) exon 2; c.35G>T; p.G12V, variant allele frequency: 20%, 630/3168 reads.

Bone marrow biopsy (CMML)

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: NGS OPv3.0

Variant(s) detected:
Mutation in NRAS (NM_002524) exon 2; c.35G>T; p.G12V, variant allele frequency: 42%, 895/2133 reads.

Case 3

First bone marrow biopsy (mixed MM/ECD bone marrow)

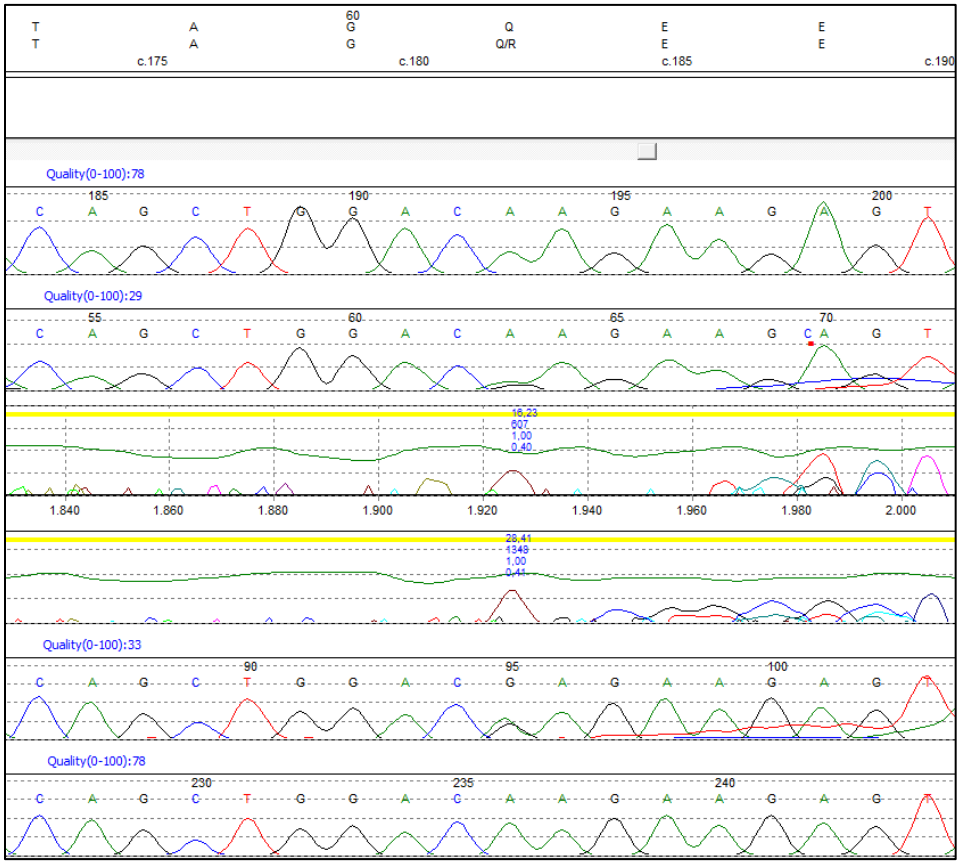
Next generation sequencing

Material: hematoxylin-stained slides, DNA isolated in duplicate
NGTS panel: Diagnostics Panel version 5.0 (DPv5.0).
Genes (exons) analyzed:
Coding sequence: CDKN2A (coverage: 98 %), PTEN (94 %) and TP53 (100 %).
Mutation hotspots: AKT1 (exon: 3), ALK (20, 22-25), APC (14), ARAF (7), BRAF (11, 15), CTNNB1 (3, 7, 8), EGFR (18-21), EZH2 (16), FBXW7 (9, 10), FOXL2 (1), FGFR1 (4, 7, 12), FGFR2 (7, 9, 12), FGFR3 (7, 9), GNA11 (4, 5), GNAQ (4, 5), GNAS (8, 9), HER2 (19-21), HRAS (2-4), IDH1 (4), IDH2 (4), KIT (8, 9, 11, 13, 14, 17), KRAS (2-4), MAP2K1 (2, 3), MET (2, 14, 19), MYD88 (5), NOTCH1 (26, 27), NRAS (2-4), PDGFRA (12, 14, 18), PIK3CA (10, 21), POLD1 (12), POLE (9, 13), RAF1 (7), RET (11, 16), RNF43 (3, 4, 9), ROS1 (38, 41), SMAD4 (3, 9, 12) and STK11 (4, 5, 8).

Variant(s) detected:
Analysis 1st DNA isolate: mutation in NRAS (NM_002524) exon 3; c.182A>G; p.Q61R, variant allele frequency: 69%, 18/26 reads
Analysis 2nd DNA isolate: mutation in NRAS (NM_002524) exon 3; c.182A>G; p.Q61R, variant allele frequency: 18%, 6/33 reads

NOTE: Number of amplicons with low coverage (<100 reads), which may cause mutations to be missed:
1st DNA isolate: 161/171 amplicons, including in *BRAF.*
2nd DNA isolate: 152/171 amplicons, including in *BRAF.*

Sanger sequencing of *NRAS* exon 3 using the 2nd DNA isolate confirmed the presence of the *NRAS* c.182A>G; p.Q61R mutation. Sanger sequencing of the 1st DNA isolate was not possible due to insufficient amount of DNA.



Left tibia biopsy (ECD sclerotic bone lesion)

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: Diagnostics Panel version 5.1 (DPv5.1).
Genes (exons) analyzed:
Coding sequence: CDKN2A (coverage: 98 %), PTEN (94 %) and TP53 (100 %).
Mutation hotspots: AKT1 (exon: 3), ALK (20, 22-25), APC (14), ARAF (7), BRAF (11, 15), CTNNB1 (3, 7, 8), EGFR (18-21), EZH2 (16), FBXW7 (9, 10), FOXL2 (1), FGFR1 (4, 7, 12), FGFR2 (7, 9, 12), FGFR3 (7, 9), GNA11 (4, 5), GNAQ (4, 5), GNAS (8, 9), HER2 (19-21), HRAS (2-4), IDH1 (4), IDH2 (4), KIT (8, 9, 11, 13, 14, 17), KRAS (2-4), MAP2K1 (2, 3), MET (2, 14, 19), MYD88 (5), NOTCH1 (26, 27), NRAS (2-4), PDGFRA (12, 14, 18), PIK3CA (10, 21), POLD1 (12), POLE (9, 13), RAF1 (7), RET (11, 16), RNF43 (3, 4, 9), ROS1 (38, 41), SMAD4 (3, 9, 12) and STK11 (4, 5, 8).
Not-coding sequence: TERT promoter.

Variant(s) detected:
Mutation in NRAS (NM_002524) exon 3; c.182A>G; p.Q61R, variant allele frequency: 37%, 457/1232 reads.

Skin biopsy (ECD xanthelasma-like skin lesion)

Next generation sequencing
Material: hematoxylin-stained slides
NGTS panel: Diagnostics Panel version 5.1 (DPv5.1).

Variant(s) detected:
Mutation in NRAS (NM_002524) exon 3; c.182A>G; p.Q61R, variant allele frequency: 37%, 262/701 reads.

Bone marrow aspirate (mixed AML/ECD bone marrow)

Next generation sequencing
Material: 6mL EDTA bone marrow aspirate
NGTS panel: NGS Illumina TruSight Myeloid Sequencing panel
Genes (exons) analyzed:

Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)	Gene	Target Region (exon)
ABL1	4–6	*DNMT3A*	full	*KDM6A*	full	*RAD21*	full
ASXL1	12	*ETV6/TEL*	full	*KIT*	2,8–11,13,17	*RUNX1*	full
ATRX	8–10,17–31	*EZH2*	full	*KRAS*	2,3	*SETBP1*	4 (partial)
BCOR	full	*FBXW7*	9–11	*MLL*	5–8	*SF3B1*	13–16
BCORL1	full	*FLT3*	14,15,20	*MPL*	10	*SMC1A*	2,11,16,17
BRAF	15	*GATA1*	2	*MYD88*	3–5	*SMC3*	10,13,19,23,25,28
CALR	9	*GATA2*	2–6	*NOTCH1*	26-28,34	*SRSF2*	1
CBL	8,9	*GNAS*	8,9	*NPM1*	12	*STAG2*	full
CBLB	9,10	*HRAS*	2,3	*NRAS*	2,3	*TET2*	3–11
CBLC	9,10	*IDH1*	4	*PDGFRA*	12,14,18	*TP53*	2–11
CDKN2A	full	*IDH2*	4	*PHF6*	full	*U2AF1*	2,6
CEBPA	full	*IKZF1*	full	*PTEN*	5,7	*WT1*	7,9
CSF3R	14–17	*JAK2*	12,14	*PTPN11*	3,13	*ZRSR2*	full
CUX1	full	*JAK3*	13				

Variant(s) detected:
Mutation in NRAS (NM_002524) exon 3; c.182A>G; p.Q61R, variant allele frequency: 44%, 464/1064 reads.