Cell-free DNA from nail clippings as source of normal control for genomic studies in hematologic malignancies

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Supplementary methods section

Nail collection:

Both patient and clinicians are provided instructions on nail collection. A nail collection kit, including new nail clippers, a sterile container and a biohazard bag are provided. Nails are commonly collected in the clinics at the time of patient consent but may be collected at home following the provided instructions, when necessary. Briefly, the patient is instructed to clean their hands and nails immediately before collection. Hands are washed with soap and water, with careful attention to the nails. Nails should be free from dirt and dry prior to nail clipping. Clinicians are instructed to check nails for any unusual appearance or discoloration and, if the nails do not appear normal, collection should be avoided. Nails should be clear of any substances including, but not limited to, polish, artificial acrylic, gels, or silky overlays. Non-cosmetic substances such as dirt, residue oils, paint, ink, dyes, etc., should be removed. When removing fingernail polish prior to collection, a non-ethanol-based polish remover such as isopropyl alcohol or acetone should be used. After thorough cleaning and drying, thin slivers from the free edge (most distal end) of the nail area are collected using the nail clipper provided, avoiding collection close to the skin, and avoiding trauma or bleeding. Ten fingernail clippings are recommended.

Nail extraction:

Nail DNA extraction was performed with the QIAamp® DNA investigator kit (Qiagen), for forensic and human identity samples. Two nail fragmentation methods were used (Figure 1A): Method 1 strictly followed manufacturer's protocol. Briefly, nail clippings (2-3, ~10mg) were manually cut into 1-2mm fragments with scissors and digested overnight with Buffer ATL, proteinase K and 1M DL-Dithiothreitol Solution (DTT) (Sigma Cat. No. 646563 10X0.5 ml), at 56℃ in a rotating incubator. When undigested particles were present after overnight incubation, additional proteinase K was added; the last 2 steps were repeated for several cycles (up to 6 days) to allow complete digestion. In method 2, nail clippings (~10mg) were pulverized following adjusted bone tissue protocols.(2, 3) Clippings were placed inside a 1.5ml microcentrifuge zirconium bead tube using high speed agitation and centrifugation with zirconium beads in a BeadBlaster™ 24 instrument (Benchmark Scientific, NJ) for 1 minute at a speed of 7.0M/S (miles per second). The resulting nail powder was spun down for 1 minute at 15000 RPM, followed by overnight digestion and incubation at 56℃ as described above. Both methods were validated for clinical use and their side-by-side performance characteristics established through a validation set to compare

quality, yield and performance. Method 1 was used clinically from January 2017 to June 2019 and method 2 from July 2019 to December 2021.

Following DNA extraction, all samples were eluted in a 60ul volume and DNA concentration was measured by Qubit fluorometer using the dsDNA HS Assay kit (ThermoFisher Scientific/Invitrogen Cat. No. QC32854). Subsets were also analyzed by the Agilent 5300 Fragment Analyzer System with the HS Small Fragment kit (DNF-477) and the HS Genomic DNA Kit (DNF-488-1000) (Agilent, Santa Clara, CA) to assess fragment profiles, following manufacturer protocols.

Supplementary table 1 - Nail samples with time of collection >120 days of tumor sample collection						
Sample	**Tumor mutations**	**nail mutations**	**Nail collection relative to tumor**	**OncoTree_Code**	**Broad disease CATEGORY**	**Detailed notes**
P-0021485-T03-IH3	9	1	-1512	MDSWP	MDS_WORKUP	Long standing history of progressive cytopenias for 5 yrs. No workup at the time of collection as patient was diagnosed with high-grade serous carcinoma at the same time. Blood sequenced as normal control detected 5 somatic mutations (PPM1D, CBL and 3 TET2 mutations at 2-15% VAF). Bone marrow sequencing identified 9 mutations including 2 TET2,5 TP53, PTEN and ETNK1 but morphologic assessment precluded by poor sample quality. A subsequent bone marrow sample (2 months later) was consistent with MDS-MLD 3% blasts.
P-0041336-T03-IH3	1	1	-806	PMFOFS	MPN	MPN with 3+ myelofibrosis and osteosclerosis at time of nail sampling
P-0014440-T11-IH3	3	3	-506	MDS	MDS	Myelodysplastic syndrome at the time of nail sampling. 3 mutations at low level (2-4%) but same level as tumor. Tumor sample is post transplant at time of recurrence with low level disease.
P-0047525-T02-IH3	6	5	-386	PCM	PCM	Emerging undiagnosed MDS co-occurring with PCM - case 2 in figure 5
P-0022623-T04-IH3	6	3	-376	PMF	MPN	MPN with 3+ myelofibrosis and osteosclerosis at time of nail sampling
P-0037705-T11-IH3	5	3	-309	TAML	AML	MDS/MPN at the time of nail sampling. Rapidly evolving into AML.
P-0039911-T02-IH3	3	1	-221	MDSRSMD	MDS	Myelodysplastic syndrome at the time of nail sampling. Tumor sample is post-transplant at time of recurrence with low level disease.
P-0031168-T01-IH3	4	3	-181	CMML	MDS/MPN	CMML at time of nail sampling
P-0067465-T01-IH3	2	1	153	MYCF	MTNN	Mycosis fungoides. Aggressive epidermotropic CD8+ T cell lymphoma. Extensive disease involving hands at the time of nail sampling
P-0055569-T01-IH3	8	1	171	DLBCLNOS	MBN	Refractory DLBCL. Nail collected at time of active disease. 11 alterations detected in the tumor biopsy in keeping with DLBCL - only TET2

						detected in the nail. Hypolobated megakaryocytes in marrow but no overt dysplasia. No follow up.
P-0019673-T02-IH3	5	5	173	MPN	MPN	MPN with 3+ myelofibrosis and osteosclerosis at time of nail sampling
P-0066809-T01-IH3	4	1	185	ECD	HDCN	Erdheim chester disease at time of nail sampling. Multiple mutations in tumor including ASXL1, MAP2K1 and SETD2. Only SETD2 detected in nail. Cell free DNA from plasma demonstrates active disease with detection of MAP2K1 mutation. No follow up
P-0036079-T01-IH3	4	1	369	AITL	MTNN	Angioimmunoblastic T cell lymphoma with RHOA, TET2, APC, and EZH2 mutations. Bone marrow hypercellular and left shifted myeloid maturation. No overt dysplasia. Only APC detected in nail
P-0052727-T01-IH3	2	1	406	RDD	HDCN	Rosai dorfman at time of nail sampling. Tumor shows BRAF mutation and DNMT3A. Only DNMT3A in nail. No follow up information
P-0042938-T01-IH3	15	3	570	PTCL	MTNN	PTCL involving stomach. Numerous mutations in PTCL. Only TET2, DNMT3A and GRIN2A present ~10% in nail. Patient is pancytopenic with bone marrow aplasia inconclusive for dysplasia. Died of disease before further work up.
P-0030761-T01-IH3	2	2	578	PTCL	MTNN	Peripheral T cell lymphoma. Long standing cytopenias but no diagnosis of MDS. Marrow without overt Dysplasia.
P-0026704-T03-IH3	4	3	672	AITL	MTNN	Bone marrow done for workup of thrombocytopenia - Mild atypia but no overt dysplasia. No follow up - patient died before further work up
P-0051195-T02-IH3	1	1	932	ECD	HDCN	Erheim Chester - no follow up

Table 1 includes 18 patients for whom collection of nails relative to the tumor was done beyond 4 months (120 days), before (-) or after (+).

In most cases, the patients had the same disease that was detected in the tumor. In 9 cases, the patient had active disease, similar to the tumor sample being sequenced. In 9 patients, nail mutations were CH type alterations that suggested the presence of an emerging disease myeloid disease. One patient had T cell lymphoma with extensive disease involving hands at the time of nail sampling.

Supplementary table 2: Stratification of cases by disease category – n=2610 cases

Category	Number of patients	Total Tumor Mutations	Mutations detected only in Tumor	Tumor mutations identified in Nail	Number of Patients without nail mutations	Number of Patients with nail mutations	Percent of patients with nail mutations in total cohort (n=2610)	Percent of patients with nail mutations in disease category	Percent of total mutations in category identified in the nail
ALAL	18	79	77	2	17	1	0.04	5.56	2.53
AML	389	1640	1498	142	326	63	2.41	16.20	8.66
BPDCN	1	2	2	0	1	0	0.00	0.00	0.00
HDCN	144	248	239	9	136	8	0.31	5.56	3.63
MCD	8	35	28	7	5	3	0.11	37.50	20.00
MDS	383	1156	1004	152	305	78	2.99	20.37	13.15
MDS/MPN	63	331	259	72	41	22	0.84	34.92	21.75
MDS WORKUP	77	167	144	23	64	13	0.50	16.88	13.77
MLNER	3	4	4	0	3	0	0.00	0.00	0.00
MPN	331	862	607	255	224	107	4.10	32.33	29.58
MPN WORKUP	65	115	103	12	56	9	0.34	13.85	10.43
BLL	114	543	534	9	107	7	0.27	6.14	1.66
HL	4	12	12	0	4	0	0.00	0.00	0.00
LATL	30	49	46	3	27	3	0.11	10.00	6.12
MBN	590	3592	3566	26	572	18	0.69	3.05	0.72
MTNN	324	1800	1731	69	296	28	1.07	8.64	3.83
PCM	61	278	267	11	56	5	0.19	8.20	3.96
TLL	5	29	29	0	5	0	0.00	0.00	0.00
Myeloid	1482	4639	3965	674	1178	304	11.65	20.51	14.53
Lymphoid	1128	6303	6185	118	1067	61	2.34	5.41	1.87
Grand Total	2610	10942	10150	792	2245	365	13.98		7.24

lymphoid categories									
MTNN and LATL	354					31	8.76	8.76	
MBN AND PCM	651					23	3.53	3.53	

Abbreviation key - Disease categories according to Oncotree classification

Acute Leukemias of Ambiguous Lineage (ALAL)
Acute myeloid leukemias (AML)
Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN)
Histiocytic and Dendritic Cell Neoplasms (HDCN)
Mast cell disease (MCD)
Myelodysplastic Syndromes (MDS)
Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN)
Myelodysplastic Syndromes workup - suspected but not diagnosed (MDS workup)
Myeloid/Lymphoid Neoplasms with Eosinophilia and Rearrangement of PDGFRA/PDGFRB or FGFR1 or with PCM1-JAK2 (MLNER)
Myeloproliferative Neoplasms (MPN)
Myeloproliferative Syndromes workup - suspected but not diagnosed (MPN work up)
B-Lymphoblastic Leukemia/Lymphoma (BLL)
Hodgkin Lymphoma (HL)
Lymphoid Atypical (LATL)
Mature B-Cell Neoplasms (MBN)
Mature T and NK Neoplasms (MTNN)
Plasma Cell Myeloma (PCM)
T-Lymphoblastic Leukemia/Lymphoma (BLL)

Supplementary table 3: 19 nail samples with mutations and VAF's similar to tumor (<2 fold difference)								
SAMPLE_ID	**Gene**	**AAchange**	Tumor Mutation VF	**interval between nail and tumor**	**Broad CATEGORY**	**called Nail MUTATION VF**	**Ratio T/N**	**Notes**
P-0007936-T06-IH3	PMS2	p.H479Q	6.4%	9	AML	9.2%	0.7	leukemia cutis
P-0026897-T01-IH3	KMT2C	p.T4218S	8.0%	19	MTNN	17.4%	0.5	PTCL with coexisting MDS. MDS mutations are subclonal in the tumor.
	TP53	p.H179Q	8.7%	19	MTNN	14.4%	0.6	
P-0030281-T04-IH3	JAK2	p.V617F	9.9%	0	MDS	5.7%	1.7	MDS with new subclonal JAK2 mutation
P-0022263-T06-IH3	MGA	p.M2780V	25.5%	64	AML	13.1%	2.0	AML with monocytic differentiation
P-0028285-T02-IH3	SRSF2	p.P95L	54.1%	3	MDS/MPN	27.2%	2.0	Bone marrow with 3+ myelofibrosis and osteosclerosis TET2 LOH
	GNB1	p.K57N	23.9%	3	MDS/MPN	14.2%	1.7	
P-0015800-T04-IH3	JAK2	p.K539L	1.0%	17	MPN	1.1%	0.9	Co-occurring CLL and MPN - 11 muts but only MPN mutations in the nail
P-0038107-T01-IH3	SF3B1	p.K700E	13.1%	13	MDS	10.2%	1.3	isolated mtuation no follow up
P-0041501-T02-IH3	TET2	p.R1216*	45.0%	87	MPN	25.3%	1.8	MDS/MPN - no marrow
	ETV6	p.A377V	49.4%	87	MPN	24.7%	2.0	MDS/MPN - no marrow
	NRAS	p.G12V	2.2%	87	MPN	1.4%	1.6	MDS/MPN - no marrow
P-0042938-T01-IH3	TET2	p.C1273Y	5.8%	570	MTNN	6.7%	0.9	PTCL involving stomach. Numerous mutations in PTCL. Only TET2, DNMT3A and GRIN2A present ~10% in nail. Patient is pancytopenic with marrow aplasia inconclusive for dysplasia. Died of disease before further work up.
P-0042185-T03-IH3	DNMT3A	p.R882H	1.0%	-10	AML	13.2%	0.1	recurrence AML low level. Nail collected at high disease level

P-0027720-T03-IH3	CALR	p.L367Tfs*46	39.3%	5	MPN	29.2%	1.3	3+ myelofibrosis and osteosclerosis TET2 LOH
	TET2	p.V1180D	40.5%	5	MPN	28.0%	1.4	
	PMS2	p.I26M	44.6%	5	MPN	25.5%	1.8	
	ASXL1	p.G646Wfs*12	36.6%	5	MPN	23.4%	1.6	
P-0014355-T13-IH3	TET2	p.N752Kfs*60	2.4%	12	MPN	1.6%	1.5	3+ myelofibrosis and osteosclerosis TET2 is subclonal in the tumor
P-0014440-T11-IH3	SRSF2	p.P95H	2.7%	-506	MDS	2.0%	1.3	myelofibrosis 2-3+ post transplant nail. Bone marrow with low level disease. Nail sample collected when blasts were 91%
	DNMT3A	p.R882H	1.6%	-506	MDS	1.3%	1.3	
P-0011024-T05-IH3	JAK2	p.V617F	61.3%	123	MPN	59.7%	1.0	Bone marrow with 3+ myelofibrosis and osteosclerosis
	FBXO11	p.P45_Q53del	39.5%	123	MPN	37.7%	1.0	
	MGA	p.V2637L	36.2%	123	MPN	27.4%	1.3	
	ASXL1	p.W898*	19.3%	123	MPN	19.3%	1.0	
	ASXL1	p.R661*	10.2%	123	MPN	8.8%	1.2	
	FBXO11	p.D161Y	10.7%	123	MPN	7.8%	1.4	
	FANCD2	p.F386V	10.8%	123	MPN	7.2%	1.5	
P-0044950-T04-IH3	DNMT3A	p.X285_splice	9.5%	-70	AML	9.6%	1.0	
P-0026778-T04-IH3	CHEK2	p.K365N	40.9%	1	MPN	24.4%	1.7	Bone marrow with 3+ myelofibrosis
	ETV6	p.H308Sfs*18	44.3%	1	MPN	22.3%	2.0	
	PTPN11	p.T73I	14.5%	1	MPN	7.4%	2.0	
P-0051394-T03-IH3	DNMT3A	p.S714C	42.9%	4	AML	23.7%	1.8	3+ myelofibrosis
	FBXW7	p.R689W	5.1%	4	AML	7.3%	0.7	
P-0053018-T03-IH3	BCOR	p.V797Lfs*10	27.8%	-3	AML	33.7%	0.8	Upper ext DVT PE double lung transplant
	U2AF1	p.S34F	18.5%	-3	AML	25.3%	0.7	
P-0066641-T02-IH3	TP53	p.N247T	2.4%	2	MDS	1.2%	2.0	no history

Supplementary Table 4 - Nail samples collected after transplant						
Tumor ID	Days_from _transplant at nail collection	Status	Donor component in Nail	Evidence of GVHD (within 5 months prior to collection)	Derm notes	GVHD organ involved
P-0017576-T01-IH3	1660	N/A	Not evaluable*	No	no nail notes	
P-0008825-T08-IH3	574	CMH	42% donor	Yes	no nail notes	skin
P-0016103-T11-IH3	174	H	All host	No	no nail notes	
P-0013519-T03-IH3	426	H	All host	No	no nail notes	
P-0016085-T03-IH3	48	H	All host	No	no nail notes	
P-0014440-T11-IH3	672	H	all host (SNP)	Yes	no nail notes	UGI
P-0016588-T19-IH3	134	H	all host	No	no nail notes	
P-0009572-T13-IH3	1190	H	all host (SNP)	No	no nail notes	
P-0023500-T06-IH3	215	CMH	8% donor	No	no nail notes	
P-0031786-T02-IH3	1322	CMH	11% donor	No	no nail notes	
P-0032163-T01-IH3	1334	H	All host	No	no nail notes	
P-0032196-T01-IH3	57	CMH	10 % donor	Yes	WNL but 5 mo after	UGI/LGI
P-0032881-T07-IH3	339	H	all host	No	no nail notes	
P-0015809-T02-IH3	2689	CMH	10% donor	No (note)	no nail notes	Chronic transaminitis suggestive of chronic GVHD - no biopsy
P-0037019-T01-IH3	68	H	all host (SNP)	Yes	no nail notes	Skin
P-0037359-T01-IH3	309	H	All Host	No	no nail notes	
P-0025105-T03-IH3	125	H	SNP all host	No	no nail notes	
P-0028818-T01-IH3	2826	CMH	27% donor	Yes	no nail notes	Skin / eye
P-0020600-T06-IH3	1621	H	all host (SNP)	No	no nail notes	
P-0027574-T09-IH3	364	H	All host	No	no nail notes	
P-0042753-T01-IH3	2752	H	all host	No	no nail notes	
P-0020933-T14-IH3	504	H	all host (SNP)	No	no nail notes	
P-0043895-T01-IH3	960	H	all host (SNP)	No	no nail notes	
P-0028179-T07-IH3	356	H	all host (SNP)	No	no nail notes	
P-0034008-T04-IH3	257	H	All Host	No	no nail notes	
P-0044711-T01-IH3	2491	H	All Host	No	WNL	

P-0039015-T07-IH3	41	H	All Host	No	nail only from 1/3/2020, brittle	
P-0037562-T03-IH3	1679	H	all host (SNP)	No	no nail notes	
P-0014446-T10-IH3	918	H	all host (SNP)	No	no nail notes	
P-0034055-T04-IH3	257	H	All Host	No	no nail notes	
P-0033046-T07-IH3	132	H	All Host	No	WNL	
P-0007826-T08-IH3	1614	CMH	9% Donor	Yes	no nail notes	Liver
P-0030284-T01-IH3	1433	H	all host (SNP)	No	no nail notes	
P-0054824-T01-IH3	42	H	all host	No	no nail notes	
P-0026085-T15-IH3	855	CMH	9% donor	yes	no nail notes	Liver
P-0052508-T03-IH3	117	H	All Host	Yes	WNL	Skin
P-0056752-T01-IH3	41	H	all host (SNP)	Yes	no nail notes	UGI
P-0051949-T01-IH3	2661	H	All host	Yes	no nail notes	Eye
P-0041558-T02-IH3	3	H	all host (SNP)	No	no nail notes	
P-0034841-T07-IH3	211	CMH	15% Donor	yes	no nail notes	Skin
P-0057334-T04-IH3	307	H	All Host	No	no nail notes	
P-0016512-T13-IH3	427	CMH	14% Donor	No	no nail notes	
P-0036198-T07-IH3	3918	H	all host (SNP)	No	no nail notes	
P-0014218-T12-IH3	960	H	all host (SNP)	No	no nail notes	
P-0038787-T09-IH3	123	H	all host	no	no nail notes	
P-0031035-T16-IH3	784	H	all host (SNP)	No	no nail notes	
P-0009129-T16-IH3	1364	H	all host (SNP)	No	no nail notes	
P-0020531-T18-IH3	880	H	all host (SNP)	No	no nail notes	
P-0065092-T01-IH3	56	CMH	5% Donor	yes	no nail notes	UGI
P-0056824-T03-IH3	257	H	all host (SNP)	No	no nail notes	
P-0061278-T05-IH3	9	H	all host (SNP)	Yes	no nail notes	UGI