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CLINICAL GENOMICS

Circulating Tumor DNA in a Breast Cancer Patient's Plasma Represents Driver Alterations in the Tumor Tissue

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Tumor tissues from biopsies or surgery are major sources for the next generation sequencing (NGS) study, but these procedures are invasive and have limitation to overcome intratumor heterogeneity. Recent studies have shown that driver alterations in tumor tissues can be detected by liquid biopsy which is a less invasive technique capable of both capturing the tumor heterogeneity and overcoming the difficulty in tissue sampling. However, it is still unclear whether the driver alterations in liquid biopsy can be detected by targeted NGS and how those related to the tissue biopsy. In this study, we performed whole-exome sequencing for a breast cancer tissue and identified *PTEN* p.H259fs*7 frameshift mutation. In the plasma DNA (liquid biopsy) analysis by targeted NGS, the same variant initially identified in the tumor tissue was also detected with low variant allele frequency. This mutation was subsequently validated by digital polymerase chain reaction in liquid biopsy. Our result confirm that driver alterations identified in the tumor tissue were detected in liquid biopsy by targeted NGS as well, and suggest that a higher depth of sequencing coverage is needed for detection of genomic alterations in a liquid biopsy.

Keywords: breast neoplasms, CDK4 amplification, circulating tumor DNA, liquid biopsy, next generation sequencing, PTEN mutation

Next generation sequencing (NGS) technology has not only revolutionized cancer research but also is currently being used to guide clinicians' decision-making for cancer treatments. Although tumor tissues from biopsies or surgery are major sources for the NGS study of primary, metastatic and resistant tumors, these serial tumor biopsies are often invasive procedures limited to certain locations and not easily acceptable in the clinic. More importantly, tissue biopsy has a severe limitation in view of the pronounced genomic and phenotypic heterogeneity of the tumor tissues [1]. To overcome the limitations of tissue biopsies or surgery, a less invasive technique capable of both capturing the tumor heterogeneity and overcoming the difficulty in tissue sampling during the course of therapy is needed. Circulating tumor DNA (ctDNA) is comprised of small fragments of DNA released from cells undergoing apoptosis or necrosis in tumor tissues [2]. Recent studies have shown that driver alterations in tumor tissues can be detected by liquid biopsy for ctDNA [3]. However, it is still uncertain whether the driver alterations in ctDNA can be detected by targeted NGS and how those related to the tissue biopsy.

A 56-year old woman was referred to the oncology

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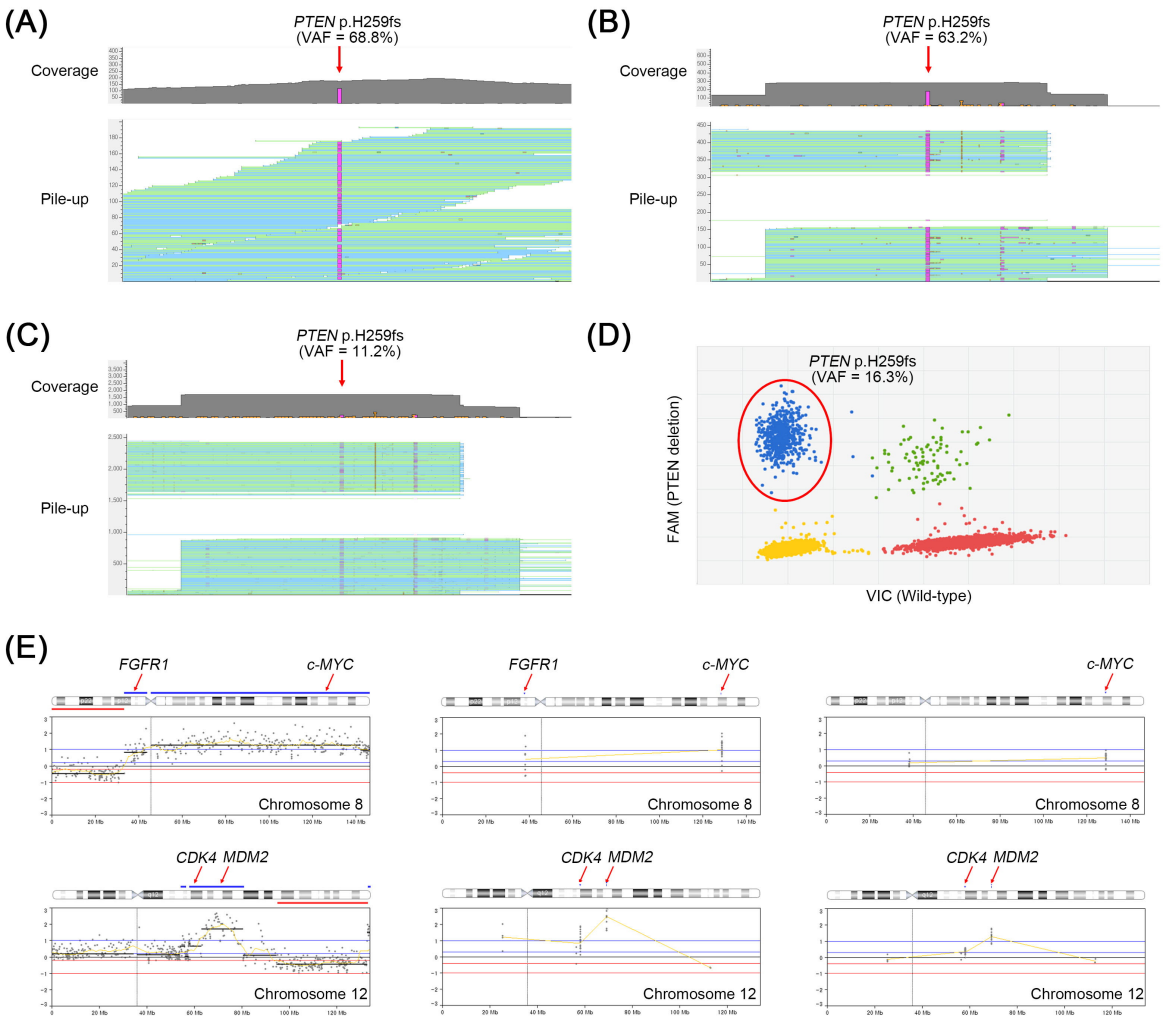

Fig. 1. *PTEN* frameshift mutation identified in a breast tumor tissue and circulating tumor DNA (ctDNA). H259fs mutation is detected by the whole-exome sequencing (WES) (A) and OncoChase targeted sequencing (B) in the breast tumor. (C) The same mutation is also detected by the OncoChase targeted sequencing in the ctDNA. (D) Validation of the *PTEN* mutation by a digital polymerase chain reaction in ctDNA. In all experiments, the *PTEN* mutation was not detected in the matched normal DNA. (E) Copy number alterations identified in breast tumor or ctDNA by next generation sequencing. Amplification of *FGFR1*, *c-MYC*, *CDK4*, and *MDM2* are detected in the tumor tissue by WES (left panel). Among them, *c-MYC*, *CDK4*, and *MDM2* amplifications are consistently detected in the tumor tissue (middle panel) or ctDNA (right panel) by OncoChase targeted panel sequencing. The x-axis represents genomic position and the y-axis represents the relative depth ratio (tumor/matched normal) in log2 scale. VAF, variant allele frequency.

department for evaluation of her right shoulder pain. She was previously diagnosed as a breast cancer 6 years ago. The patient was treated with quadrantectomy along with axillary node dissection (invasive ductal carcinoma, pT2N0M0, estrogen receptor [ER] positive, progesterone receptor positive and human epidermal growth factor receptor 2 [HER2] negative), adjuvant radiation of the left breast, and adjuvant toremifen (an oral selective ER modulator) for 5 years. Seven months after the termination of adjuvant toremifen, the patient a pathologic fracture of right distal clavicle, multiple bone, lung and liver metastases (Supplementary Fig. 1). During 3 years of the systemic treatment, the patient had progression of chest, liver and bone metastases (Supplementary Fig. 2).

To identify the genetic alterations in the tumor tissue, we performed whole-exome sequencing (WES) for the cancer tissue metastasized to femur along with her matched normal DNA form peripheral blood. Generation and processing of the sequencing data were performed as previously described [4]. In the WES, a total of 53 nonsilent somatic mutations and 23 copy number alterations (CNAs) were detected (Supplementary Tables 1 and 2). Among them, *PTEN* p.H259fs*7 frameshift mutation (Fig. 1A) as well as *FGFR1*, *c-MYC*, *CDK4*, and *MDM2* amplifications (Fig. 1E) were identified as

driver alterations in this tumor.

To validate these, the metastatic tumor and matched normal samples of the patient were re-analyzed by a targeted NGS. Targeted NGS was performed using the OncoChase Cancer Panel v0.9 (ConnectaGen, Seoul, Korea) consisting of 78 well-characterized cancer genes (Supplementary Table 3) with Ion PGM Dx system (Thermo Fisher Scientific, Waltham, MA, USA). Coverage of depth was 416× for the metastatic tumor sample and 754× for the normal sample. In the OncoChase analysis, all five driver alterations previously identified in the WES were consistently detected (Fig. 1B and 1E). Of note, variant allele frequency (VAF) of *PTEN* frameshift mutation detected by WES (68.8%) were almost similar to that detected by OncoChase (63.2%), suggesting the OncoChase Cancer Panel may be reliable for the detection of driver alterations.

Next, we analyzed the plasma DNA (liquid biopsy) of the patient using the same cancer panel (OncoChase Cancer Panel v0.9). Coverage of depth was 1,213× for the liquid biopsy sample. The *PTEN* frameshift mutation initially identified in the tumor tissue was also detected in her liquid biopsy with a VAF of 11.2% (Fig. 1C). This mutation was subsequently validated by digital polymerase chain reaction (Fig. 1D). The ctDNA also harbored *c-MYC, CDK4* and *MDM2* amplification but signal intensities of the CNAs were relatively lower than those of the tissue biopsy (Fig. 1E). One CNA (*FGFR* amplification) identified in the tumor tissue was not detected in the ctDNA.

Comprehensive review of the hormone status (ER positive) and genetic alteration (*CDK4* amplification) status strongly suggested the use of CDK4/6 inhibitors such as palbociclib [5] combined with aromatase inhibitor [6]. However, due to her dismal hepatic function, palbociclib was not administered, and she passed away after 52 months of cancer recurrence. In this study, we showed an example that driver alterations identified in the tumor tissue were detected in liquid biopsy by targeted NGS as well. The low VAF of mutation and attenuated CNA signals in the liquid biopsy compared to the tissue biopsy suggest that a higher depth of sequencing coverage is required for detection of genomic alterations in a liquid biopsy than in a tissue biopsy.

Supplementary materials

Supplementary data including three tables and two figures can be found with this article online at http://www.genominfo.org/src/sm/gni-15-48-s001.pdf.

Acknowledgments

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SUPPLEMENTARY INFORMATION

Circulating Tumor DNA in a Breast Cancer Patient's Plasma Represents Driver Alterations in the Tumor Tissue

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http://www.genominfo.org/src/sm/gni-15-48-s001.pdf

Supplememtary Table 1. A list of non-silent somatic mutations identified in a breast tumor by WES

Gene	Genomic position[a]	Ref	Alt	Amino acid change	Exonic function
ATAD3B	chr1:1421523	A	G	p.I333V	Missense
HIST2H2AC	chr1:149858602	C	G	p.F26L	Missense
FLG2	chr1:152327810	C	A	p.A818S	Missense
RYR2	chr1:237778051	C	A	p.L1875M	Missense
ZBTB18	chr1:244217493	G	T	p.K130N	Missense
CLCA2	chr1:86920950	G	A	p.E858K	Missense
TTN	chr2:179596168	A	C	p.N4531K	Missense
TTN	chr2:179659713	G	A	p.A394V	Missense
FZD5	chr2:208632105	G	C	p.F453L	Missense
C2orf71	chr2:29295753	A	G	p.F459L	Missense
EAF1	chr3:15473643	A	G	p.Q83R	Missense
MAP6D1	chr3:183535851	C	G	p.K150N	Missense
LAMB2	chr3:49168547	G	A	p.R251C	Missense
ABHD14A	chr3:52012068	G	A	p.R84H	Missense
FOXP1	chr3:71026845	A	T	p.Y459*	Nonsense
MANBA	chr4:103560968	C	A	p.R639L	Missense
OSTC	chr4:109584406	T	C	p.L150S	Missense
INPP4B	chr4:142950014	G	A	p.A899V	Missense
DCAF4L1	chr4:41983994	G	A	p.R62Q	Missense
SLC4A9	chr5:139747461	C	T	p.R715C	Missense
PCDH12	chr5:141335297	C	T	p.R707H	Missense
MROH2B	chr5:41009477	C	A	p.E1109*	Nonsense
HTR1A	chr5:63256294	T	G	p.K418T	Missense
MCM9	chr6:119136331	G	C	p.H1030D	Missense
PKHD1	chr6:51750729	T	G	p.D2384A	Missense
TRRAP	chr7:98550994	G	A	p.G1865R	Missense
TEX15	chr8:30706046	T	A	p.N163I	Missense
OR13C2	chr9:107367629	G	A	p.L94F	Missense
SEC16A	chr9:139361484	C	CG	p.P1106fs	Frameshift
ENTPD8	chr9:140332517	G	A	p.A49V	Missense
GATA3	chr10:8097760	G	C	p.D48H	Missense
PTEN	chr10:89717748	TC	T	p.H259fs	Frameshift
PLEKHA7	chr11:16847842	G	A	p.R390W	Missense
AHNAK	chr11:62295933	T	C	p.T1986A	Missense
SLC4A8	chr12:51873974	C	T	p.R738W	Missense
SLC4A8	chr12:51873986	A	G	p.M742V	Missense
PTPRB	chr12:70965654	G	T	p.T1019N	Missense
BBS10	chr12:76741388	C	G	p.W126S	Missense
NANOGNB	chr12:7917904	C	T	p.T8M	Missense
NANOGNB	chr12:7917942	A	G	p.R21G	Missense
ATP11A	chr13:113527939	G	A	p.G1037E	Missense
UNKL	chr16:1444138	C	T	p.V311I	Missense
TNRC6A	chr16:24826564	T	C	p.I1590T	Missense
BCO1	chr16:81298374	A	T	p.I201F	Missense
SLC13A5	chr17:6607363	GC	G	p.G127fs	Frameshift
TXNDC2	chr18:9887329	T	C	p.S285P	Missense
TBXA2R	chr19:3594967	G	A	p.A364V	Missense
ZNF850	chr19:37239099	G	C	p.T916S	Missense
ZNF573	chr19:38229692	A	G	p.S479P	Missense
ZNF343	chr20:2472692	A	G	p.S115P	Missense
ZNF343	chr20:2472699	T	C	p.I112M	Missense
ABHD16B	chr20:62494200	G	A	p.R436Q	Missense
RPL10	chrX:153626864	G	A	p.G2S	Missense

WES, whole-exome sequencing.

[a]UCSC GRCh37/hg19.

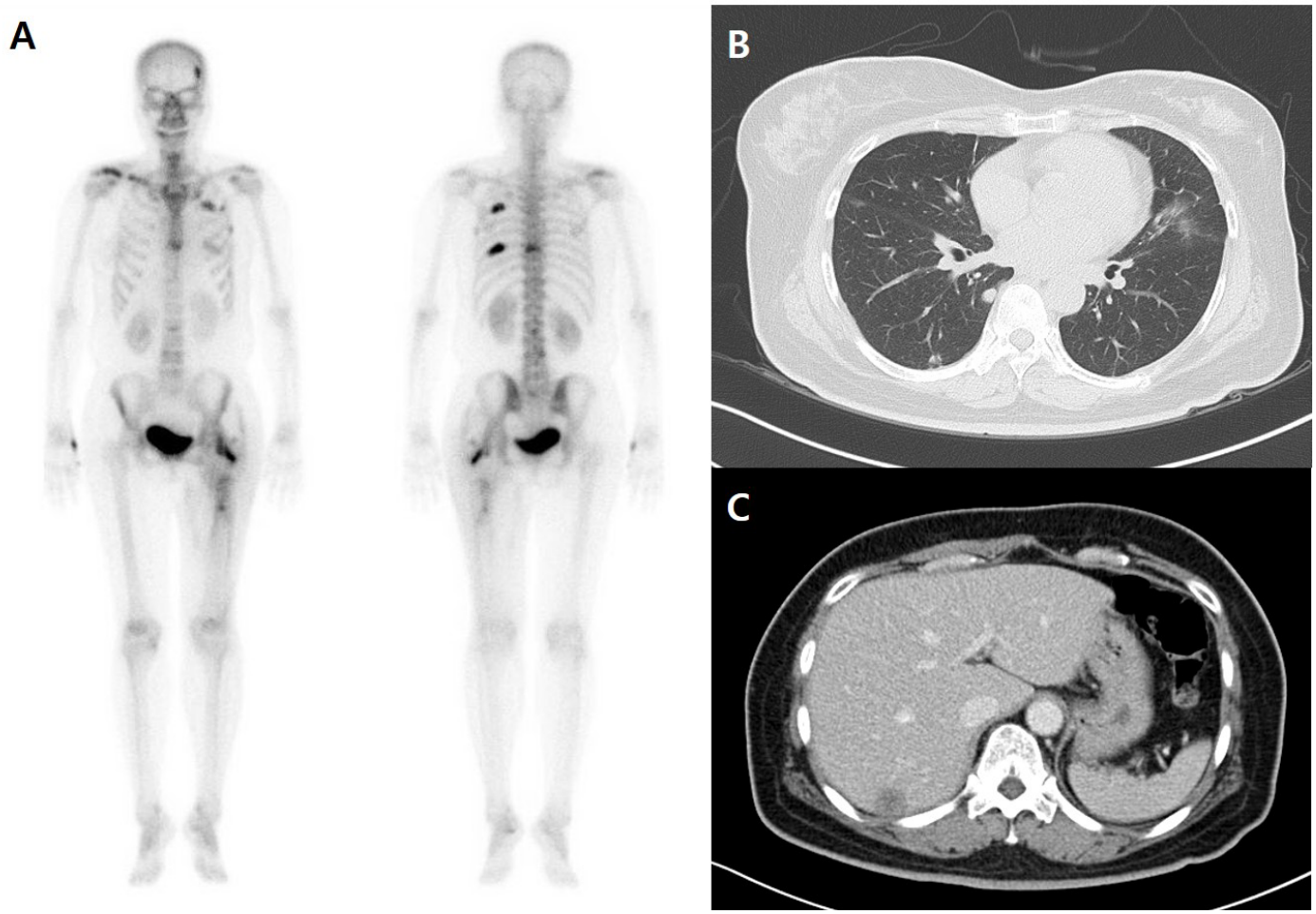
Supplementary Table 2. A list of copy number alterations identified in a breast tumor by WES

Chromosome	Start	End	Event	Length	Cancer gene
chr1	127,420,311	249,250,621	Gain	121,830,311	*MDM4*
chr3	36,534,627	69,244,337	Loss	32,709,711	
chr4	164,087,858	191,154,276	Loss	27,066,419	
chr7	0	41,804,809	Gain	41,804,810	
chr7	51,258,647	59,900,000	Gain	8,641,354	*EGFR*
chr7	61,967,549	75,174,027	Gain	13,206,479	
chr7	75,174,027	151,884,364	Loss	76,710,338	
chr7	151,884,364	159,138,663	Gain	7,254,300	
chr8	0	33,371,138	Loss	33,371,139	
chr8	33,371,138	43,827,943	Amplification	10,456,806	*FGFR1*
chr8	45,600,000	146,364,022	Amplification	100,764,023	*MYC*
chr12	53,900,617	56,077,919	Gain	2,177,303	
chr12	57,592,078	80,603,169	Amplification	23,011,092	*CDK4, MDM2*
chr12	94,543,468	132,626,899	Loss	38,083,432	
chr12	132,626,899	133,851,895	Amplification	1,224,997	
chr14	67,389,335	107,349,540	Loss	39,960,206	
chr16	0	34,640,903	Gain	34,640,907	
chr17	0	24,000,000	Loss	24,000,001	*TP53*
chr19	0	19,770,314	Loss	19,770,315	*STK11*
chr20	0	25,734,258	Gain	25,734,259	
chr20	27,500,000	47,364,269	Gain	19,864,270	
chr20	47,364,269	57,415,391	Amplification	10,051,123	
chr20	57,415,391	63,025,520	Gain	5,610,130	

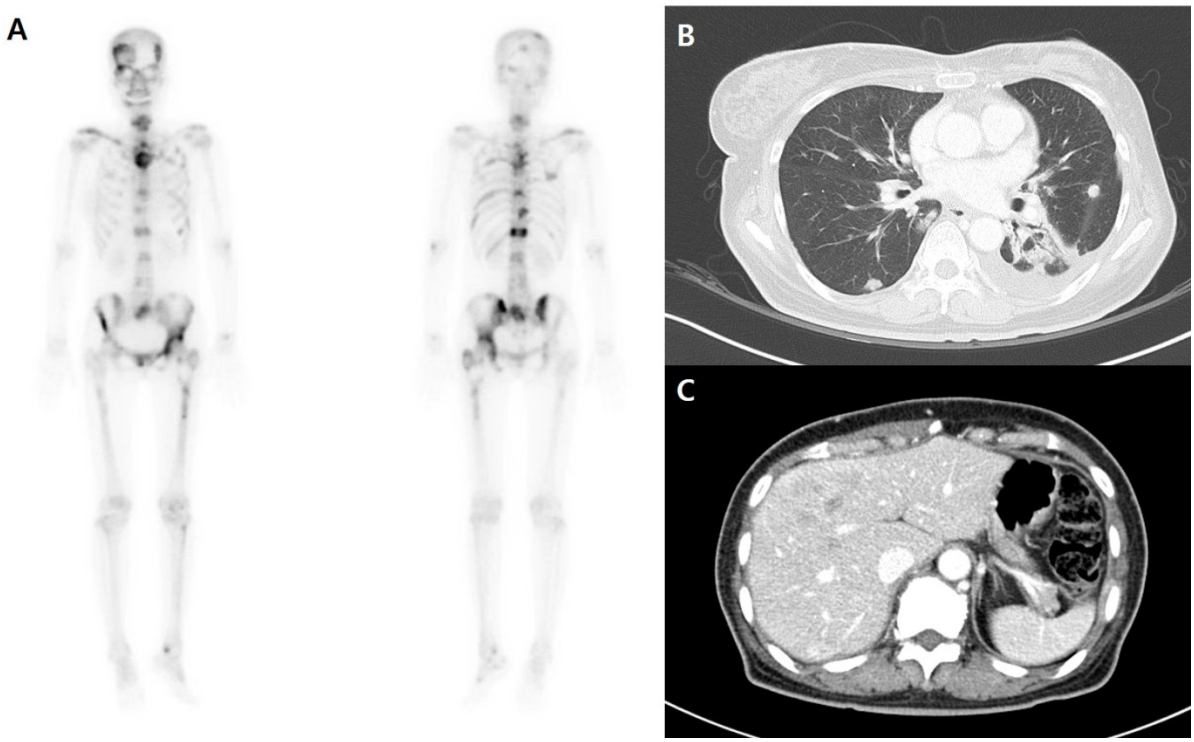
WES, whole-exome sequencing.

Supplementary Table 3. Seventy-eight cancer genes for OncoChase cancer panel analysis

MTOR	*CTNNB1*	*SMO*	*ATM*	*ERBB2*
ARID1A	*RHOA*	*BRAF*	*SDHD*	*BRCA1*
MPL	*PIK3CA*	*EZH2*	*KRAS*	*SPOP*
JAK1	*FGFR3*	*FGFR1*	*ERBB3*	*SMAD4*
NRAS	*PDGFRA*	*MYC*	*CDK4*	*STK11*
MCL1	*KIT*	*JAK2*	*MDM2*	*GNA11*
DDR2	*KDR*	*CDKN2A*	*PTPN11*	*JAK3*
DNMT3A	*FBXW7*	*GNAQ*	*FLT3*	*CCNE1*
ALK	*TP53*	*ABL1*	*BRCA2*	*SRC*
XPO1	*APC*	*NOTCH1*	*RB1*	*AURKA*
NFE2L2	*NPM1*	*RET*	*NKX2-1*	*GNAS*
IDH1	*ESR1*	*PTEN*	*AKT1*	*U2AF1*
ERBB4	*RAC1*	*PLEKHS1*	*MAP2K1*	*MAPK1*
VHL	*EGFR*	*FGFR2*	*IDH2*	*MED12*
RAF1	*CDK6*	*HRAS*	*CDH1*	*MLH1*
MET	*CCND1*	*TERT*		



Supplementary Fig. 1. Disease status at the time of cancer recurrence. Bone scan shows multiple bone metastases involving spine, sacrum and rib (A). Chest computed tomography (CT) scan shows multiple lung metastases (B), and abdomen CT scan reveals liver metastasis (C).



Supplementary Fig. 2. Disease status at the time of whole exome sequencing for breast cancer. Progression of bone metastases involving whole spine, both femur shaft and pelvic bone was detected by bone scan (A). Chest computed tomography (CT) scan shows progressive lung metastases (B) and abdomen CT scan shows progression of liver metastases (C).