의학 석사 학위논문

전이성 유방암환자에서

차세대 염기서열분석의 임상 적용

Clinical Implication of

Next Generation Sequencing

for Patients with Metastatic Breast Cancer

2020 년 8 월

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Clinical Implication of Next Generation Sequencing for Patients with Metastatic Breast Cancer

by Go-un Woo

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Approved by Thesis Committee:

Professor _____ Chairperson

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Abstract

Clinical Implication of Next Generation Sequencing for Patients with Metastatic Breast Cancer

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Next-generation sequencing (NGS) is a method that uses massive parallel sequencing and analyzes numerous variations faster than conventional sequencing. Because of NGS, many advances have been made in cancer treatment through the discovery of disease-related mutations and treatments for them. Common genetic variations in breast cancer of Korean patients have been previously identified, leading to investigations of how this genetic information can be used to treat metastatic breast cancer in clinical practice. In this study, sequencing results and medical records of 182 patients with primary or metastatic breast cancer who underwent in-hospital target sequencing were retrospectively analyzed from October 2016 to March 2020. A total of 1,428 variants were identified in 243 genes, and the median number of non-synonymous mutations per sample was 7 (0-22). The most common mutations in all samples were found in TP53 (59.8%) and PIK3CA (31.2%). Frequently altered genes differed according to the subtype; ERBB2 amplification (80%) was commonly found in human epidermal growth factor receptor 2 (HER2)-positive subtype, while TP53 (66.1%), ROS1 (19.4%), KMT2D (17.7%), and BRCA1 (14.5%) mutations

were frequently detected in triple-negative breast cancer. Druggable target was detected in 61.5% (112/182) of the cases. Moreover, among 124 patients with metastatic breast cancer, sequencing results were clinically applicable in 21.8% (27/124) of them, and 4.3% (5/124) of these patients changed treatment decisions using NGS results, with some patients notably benefitting.

In conclusion, through the NGS-based pan-cancer panel, the mutational landscape of breast cancer patients was elucidated, and the practical value in their treatment was identified.

Key words: Breast cancer, NGS, genetic alteration

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Introduction

Breast cancer is the most common cancer in women and 2.1 million new cases were diagnosed in 2018, worldwide[1]. In Korea, 22,395 patients were newly diagnosed with breast cancer, making it the 5th frequent cancer (9.6%) in population, and the most common cancer in women (20.3%)[2]. Survival rates vary widely among countries, but this disease is the leading cause of cancer-related deaths among women in most countries[1]. In 2018, 2,473 people died of breast cancer globally, and breast cancer was sixth-ranked (8.1%) cause of cancer death[3]. In addition, both the incidence and mortality of this diseases is increasing worldwide [4].

The history of systemic therapy in breast cancer began in 1895 by a surgeon named Thomas Beatson. This British surgeon performed bilateral oophorectomy in a young woman with advanced breast cancer, and the patient experienced a complete regression of the tumor[5]. However, not all patients benefited from oophorectomy. Stanley Boyd reported a case series of patients undergoing oophorectomy for breast cancer. Only a third of the patients benefited from oophorectomy, and in the majority of cases, the response lasted 6-12 months[6]. Elwood Jenson reported that estrogen receptor (ER)-rich breast cancers were likely to respond to endocrine ablation[7]. Among the treatments targeting ER that have been attempted since then, tamoxifen, a selective estrogen receptor modulator that targeted the ER, was approved by the US Food and Drug Administration (FDA)[8]. Meanwhile, it has been reported that the human epidermal growth factor receptor 2 (HER2) gene is overexpressed in some breast cancer patients, and trastuzumab, targeted agent for HER2 has significantly improved the treatment outcomes for HER2+ breast cancer[9]. Through this process,

it became known that breast cancer is a heterogeneous complex of disease, and immunohistochemistry (IHC) markers such as ER, progesterone receptor (PR), and HER2 began to be used for patient's management and predict prognosis [10].

In 2000, Perou et al. described the intrinsic subtypes of breast cancer using gene expression profiles of breast cancer surgical tissue[11]. These molecular subgroups were identified, as luminal A, B, HER2-enriched, basal-like and normal breast-like groups[12]. It is a challenging issue to use molecular subtyping based on gene expression profile because of feasibility issues including high cost and slow turn-around-time. Therefore, clinical classification using immunohistochemistry was used in daily practice. Based on the immunohistochemistry stain, luminal A breast cancer is hormone receptor + (HR+), HER2-, and has low levels of proliferative index Ki-67. Luminal B breast cancer is HR+, either HER2+ or HER2 - with high levels of Ki-67. HER2-enriched breast cancer is HR- and HER2+. Triple-negative breast cancer (TNBC) is HR- and HER2-. This classification has shown prognostic value and usefulness for predicting treatment response[13]. Currently, treatment of breast cancer is determined based on this molecular subclassification.

Cytotoxic chemotherapy accounts for a large part of the treatment of metastatic breast cancer, but targeted therapy plays an important role depending on the subtype. In breast cancer with HR+/HER2-, endocrine treatment should be considered, and if a patient responds to one endocrine treatment and then progresses, the patient could respond to subsequent endocrine treatment. Endocrine treatment can be divided into four categories. The first is the administration of antiestrogen agents, such as selective estrogen receptor modulators including tamoxifen or toremifen. The second

method involves estrogen deprivation, administration of aromatase inhibitor (AI) to inhibit peripheral conversion of androstenedione to estradiol in postmenopausal woman, or luteinizing hormone-releasing hormone agonist to inhibit the production of the estradiol from ovary in premenopausal woman, and the third is the administration of selective estrogen receptor degradator, fulvestrant which cause degradation of ER in breast cancer. The fourth category is sex hormone progestin or high dose estrogen, which is rarely used clinically. Endocrine treatment could be used until visceral crisis occurs or the patient reaches a endocrine resistance. The majority of HR+ breast cancer patients develop resistance to endocrine treatment. The PI3K/mTOR pathway is frequently altered in HR+ breast cancer and has been implicated in resistance to endocrine treatment [14, 15]. In HR+ breast cancer patients who failed nonsteroidal AI, it was confirmed that survival gain was obtained with steroidal AI exemestane with mTOR inhibitor everolimus combination treatment. [16]. PIK3CA mutations exist in a large number of HR+ breast cancer patients, which induce hyperactivation of the PI3K pathway promotes estrogen-independent growth of HR+ breast cancer cells[17]. The combination of PI3K inhibition and endocrine treatment showed clinical benefit in HR+ breast cancer patients with PIK3CA mutation[18]. The growth of HR+ metastatic breast cancer is dependent on cyclin D1, a direct transcriptional target of ER, and cyclin D1 activates cyclin-dependent kinase 4/6 (CDK4/6) resulting in G1-S phase transition[19]. CDK4/6 inhibitors prevent the proliferation of cancer cells by selectively inhibiting CDK 4/6 in the G1 cell cycle that regulates cancer cell division and growth[20]. Another resistance mechanism of endocrine treatment is the ESR1 gene mutation,

which encodes ER protein[21]. ESR1 mutations have been described in 9%–40% of patients with advanced HR+ breast cancer resistance to aromatase inhibitors, and ESR1 mutation is a biomarker for poor response to AI[22]. However, fulvestrant has shown similar efficacy in patients with or without an ESR1 mutation, and other selective ER degraders (SERDs) are being developed to overcome AI resistance in ESR1 mutant breast cancer. FGFR1 amplification is also a mechanism of resistance to endocrine treatment, and is significantly correlated with inferior survival outcome in HR+ breast cancer[23]. In particular, breast cancer patients with aberrant FGFR also showed resistance to CDK4/6 inhibitors[24], and several FGFR inhibitors have been investigated in clinical trials.

HER2 is one of the receptor tyrosine kinases on the cell surface that activates intracellular signaling through receptor dimerization and is involved in cell proliferation, survival, invasion, and angiogenesis[25]. HER2 is normally overexpressed in 20%–25% of breast cancers by HER2 gene amplification[26], and this accelerates breast cancer cell growth, invasion and metastasis. In HER2+ breast cancer, introduction of trastuzumab, a monoclonal antibody that binds to the HER2 extracellular domain IV, dramatically improves survival and has become the mainstay of treatment[27]. Pertuzumab, a monoclonal antibody, binds to the HER2 extracellular domain II and prevents heterodimerization of HER2 with HER3 or other dimerization. Ttrastuzumab and pertuzumab in combination with cytotoxic chemotherapy has shown synergistic effects and prolonged survival[28]. Lapatinib is an intracellular tyrosine kinase inhibitor of HER1 and HER2, that has shown efficacy in combination with cytotoxic chemotherapy for patients with trastuzumab

resistance[29]. Trastuzumab emtansine is an antibody drug conjugate that combines trastuzumab with chemotherapeutics, DM-1. The chemotherapeutics DM-1 releases toxic effects after internalization by binding to the HER2 receptor. With this mechanism, T-DM1 demonstrated efficacy in patients previously treated with trastuzumab[30]. Another antibody drug conjugate, trastuzumab deruxtecan (DS-8201) showed anti-tumor activity based on its high potency, including efficacy against low HER2-expressing tumors[31].

HER2 targeted agents in breast cancer has primarily targeted the HER2 amplification. However, there are few breast cancer patients with non-amplified but mutated HER2, and targeted treatment for these patients is being studied. Neratinib is an irreversible tyrosine kinase inhibitor of pan-HER (HER1, HER2, HER4) and inhibits the PI3K/Akt/MAPK pathway[32].

Treatment options are limited for TNBC, and it was previously thought that there was no druggable target. Therefore, cytotoxic chemotherapy is the mainstay of treatment for TNBC. However, TNBC is a group of heterogeneous diseases, and some studies have described specific targets for some TNBC's.

BRCA mutations (including both germline and somatic mutations) are found in up to 20-30% of TNBC patients[33] and 14% of HR+ patients. Poly(ADP-ribose) polymerase (PARP) is a DNA repair enzyme that maintains genome stability, DNA repair, and cell cycle progression and apoptosis[34]. In BRCA mutant breast cancer, BRCA mutation renders an impaired DNA repair mechanism, making it sensitive to PARP inhibition[35]. PARP inhibitors, olaparib and talazoparib showed better progression free survival compared with single agent chemotherapy in germline

BRCA1/2 mutant breast cancer. Germline BRCA1/2 mutation was confirmed using MyRIAD genetics Sanger sequencing platform as companion diagnostics in the clinical trial. In Korea, the germline BRCA test is mainly performed by Sanger sequencing in the clinical diagnostic laboratory department of individual hospital and it takes 4 – 6 weeks. Although, it is very important to know the germline BRCA mutation status for metastatic breast cancer, the insurance reimbursement was limited to the specific conditions in Korea: 1) breast cancer diagnosed at age < 40 years without a family history, 2) breast cancer, ovarian cancer, metastatic prostate cancer or pancreatic cancer family history within 3rd degree relatives, 3) patients with breast cancer and ovarian cancer or pancreatic cancer diagnosed simultaneously or seqeuentially, 4) male breast cancer, 5) bilateral breast cancer, and 6) triple-negative breast cancer diagnosed at age < 60 years. Considering the ratio of BRCA mutations in TNBC patients, this limitation is a serious pitfall in TNBC treatment. Beyond BRCA mutation, as TNBC has been shown to be enriched for homologous-recombination repair defects[36], multiple other DNA-damage response (DDR) inhibitors are being developed.

Despite a variety of treatments, the development of new drugs in the field of breast cancer remains an important issue. Therefore, the importance of detecting rare mutations and linkages of clinical trial registration have also emerged. Genetic testing is time-consuming and costly, making it challenging to detect rare mutations. Meanwhile, the emergence of next-generation sequencing (NGS) was a breakthrough. By processing multiple DNAs through parallel sequencing, NGS detects multiple genes simultaneously with reduced reporting time and cost[37].

With these benefits, NGS identifies potentially actionable targets, and in some cases, these results help patients apply for treatment or participate in clinical research[38]. In breast cancer, NGS testing reports on mutational landscapes[39-41], revealed that a mutation in a specific gene was associated with prognosis[42], and various drug developments are underway. In Korea, the usefulness of cancer sequencing in various carcinomas has been acknowledged, and NGS has been approved as a partial benefit in recent years, and it has been actively implemented. Our center has experimentally introduced and conducted NGS since 2016, and it has been commercially available through continuous improvement. By including druggable targets that can be linked to clinical trials in our center, a panel that is useful and more closely related to clinics was established.

Objectives

The primary objective of this study was to find out the frequency and pattern of genetic alterations in metastatic breast cancer cohort using targeted sequencing based pan-cancer panel. The secondary objectives of this study include exploring the potential clinical effect of targeted sequencing based multigene cancer panel in each subtype of metastatic breast cancer patients and application to clinical practice.

Materials and Methods

Patients and sample collection

Our center had developed a targeted gene sequencing (TGS) panel since 2016. During the development process to validate the panel, the TGS named SNUH-FIRST pan-cancer panel was conducted. Among patients treated with malignancy at the medical oncology department of Seoul National University Hospital (SNUH), Seoul, Republic of Korea, sequencing was conducted in patients determined by their physician. After obtaining informed consent, primary or metastatic breast cancer tissues acquired from surgery or biopsy were sequenced by the SNUH-FIRST pan-cancer panel. If druggable alteration was detected, the patient received appropriate targeted agents or were connected to appropriate clinical trials.

Patients who underwent TGS with breast cancer from October 2016 to March 2020 at SNUH were retrospectively reviewed. Sequencing results and clinicopathologic characteristics were reviewed. The current study was conducted according to the Declaration of Helsinki and approved by the Institutional Review Board of SNUH (IRB No. H-1509-047-702).

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DNA sequencing and data analysis

DNA sequencing was performed using SNUH-FIRST pan-cancer panel v2, v3, and v3.1, which consists of 225 cancer-associated genes (Table 1). Genomic DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) or fresh tumor tissue using the ReliaPrep TMFFPE gDNA Miniprep system (Promega, Madison, WI,

USA), and fragmented using a Covaris Sonicator (Covaris, Woburn, MA, USA). Target regions were captured by SNUH-FIRST pan-cancer panels v2, v3, and v3.1, including 225 cancer-associated genes (Table 1). Target exons were amplified by ligation-mediated PCR and subsequently sequenced on the Illumina HiSeq2500 (Illumina Inc., San Diego, CA, USA).

Table 1. Target genes included in the SNUH-FIRST pan-cancer panel

Panel version	SNV/INDEL/CNV	Fusion	Other
Common genes included in version 2, version 3, version 3.1	AKT1, AKT2, AKT3, ALK, APC, AR, ARAF, ARID1A, ATM, ATR, AURKA, AURKB, AURKC, AXL, BAP1, BCL2, BRAF, BRCA1, BRCA2, CCND1, CCND2, CCND3, CCNE1, CDK4, CDK6, CDKN1B, CDKN2A, CDKN2B, CHEK2, CREBBP, CSF1R, CTNNB1, DDR1, DDR2, DPYD, EGFR, EP300, ERBB2, ERBB3, ERBB4, ESR1, EWSR1, FBXW7, FGF19, FGF23, FGFR1, FGFR2, FGFR3, FGFR4, GNAQ, GNAS, HRAS, IDH1, IDH2, IGF1R, IGF2, JAK2, JAK3, KDR, KEAP1, KIT, KMT2D, KRAS, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MAP3K4, MAPK1, MAPK8, MDM2, MET, MSH6, MTOR, MYC, NF1, NF2, NFE2L2, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NRAS, NRG1, NTRK1, NTRK2, NTRK3, PDGFB, PDGFRA, PDGFRB, PIK3CA, PTEN, RAD50, RB1, RET, RICTOR, RIT1, RNF43, ROS1, SMAD4, SMARCA4, SOX2, SRC, STK11, SYK, TERT, TOP2A, TP53, TP63, TSC2, UGT1A1	ALK, AXL, BRAF, EGFR, EWSR1, FGFR1, FGFR3, NRG1, NTRK1, NTRK2, NTRK3, PDGFRB, PPARG, RET, ROS1, SS18	TERT (for promoter mutation), MET (for exon 14 skipping)
Genes included in version 2	ABL2, CBFB, CBL, CDH1, CDK11B, CDKN2C, CEBPA, DNMT3A, DOT1L, EPHA3, FGF10, FGF14, FGF3, FGF4, FGF6, FLT1, FLT3, FLT4, FOXL2, GNA11, HDAC9, HGF, MDM4, MPL, NEK2, NPM1, PIK3CB, PIK3CD, PIK3R1, PIK3R2, PPARG, RBM10, RSPO1, SDK1, SMG1, SS18, TPMT		
Genes included in version3, version 3.1	ABL1, BTK, CDK1, CDK12, CDKN1A, CHEK1, DICER1, EIF1AX, EMSY, EPCAM, ERCC2, EZH2, FAM175A, FANCA, FANCC, FANCD2, FANCG, FANCI, FANCL, FANCM, FOXA1, GNB2L1, HDAC1, IGFBP3, INPP4B, IRF1, JAK1, JUN, KDM5C, KDM6A, LATS1, LATS2, MCL1, MLH1, MRE11A, MSH2, MUTYH, MYCN, PAK2, PALB2, PARP1, PARP2, PBRM1, PMS2, POLD1, POLE, POLQ, PPP2R2A, PRKCB, RAD21, RAD51, RAD51B, RAD51C, RAD51D, RAD54L, RELA, RHEB, RPTOR, SDHB, SETD2, SMARCB1, SPOP,	ERG, ETV1, FGFR2, NUTM1, STAT6, TFE3	

	SQSTM1, STAT1, SUMO1, TSC1, TSHR, VHL, XRCC2, ZBTB16		
Genes included in version 3.1 only	BARD1, BRIP1		

SNV was called by MuTect v.2 with a Bayesian algorithm, INDEL was identified by IndelGenotyper v.36. 3336[43, 44]. Called variants were annotated using ANNOVAR[45]. SNV with total depth ≥ 10, allele depth ≥ 3, allele frequency ≥ 5% (if hotspot, 1%), and INDEL with total depth ≥ 10, allele depth ≥ 3, allele frequency ≥ 10% (if hotspot, 5) was selected. SNV errors due to 8-oxoG artifacts were excluded by the OxoG filter[46].

CNV was obtained using CNVkit software[47], and copy number segment ratios of tumor and pooled normal samples were compared. Amplifications were called with ≥ 6 copies and homozygous deletions at 0 copies. Structural variants including translocations, inversions, and large deletions were called by DELLY v.0.7.2[48], and annotated using ANNOVAR and filtered by the in-hospital tool[45], and reviewed by the Integrative Genomics Viewer (v2.3.6)[49].

Breast cancer subtypes

To determine the sample subtype, IHC of ER, PR, and HER2 in the tissue used for sequencing was reviewed. ER and PR positivity were defined as ≥ 1%, and HER2 positivity was defined as IHC 3+ (strong membranous staining in 10% of cells) and/or HER2 gene amplification (HER2:CEP17 gene copy ratio ≥ 2) using fluorescent in situ hybridization (FISH)[50]. In cases of HER2IHC 2+, HER2 gene amplification testing by FISH (FISH) was performed.

Interpretation of alterations

The reported genetic alterations were compared according to the subtype of breast cancer or tissue acquisition site.

To investigate the potential differences in our data and western breast cancer data, the public datasets from “the SAFIR01, SAFIR02, SHIVA, or Molecular Screening for Cancer Treatment Optimization (MOSCATO) prospective trials” and “the metastatic breast cancer project” was compared[51].

Detected genetic alterations were annotated according to the database OncoKB[52]. In the OncoKB knowledge base, the genetic alteration are classified into 4 levels. Level 1 included genetic alterations that are FDA-recognized biomarkers. Level 2 includes genetic alterations that are biomarkers recommended as standard care by the National Comprehensive Cancer Network (NCCN). Level 3 includes genetic alterations that predict response to investigational agents in clinical trials. Level 4 includes genetic alterations that have hypothetical therapeutic implications based on preliminary, non-clinical data. In patients with actionable mutation, following treatment and their responses were identified.

Results

Baseline characteristics

Altogether, 189 samples from 182 patients were eligible for analysis. The median age of the analyzed patients was 50 years (range: 26 to 84 years), 124 (68.1%) patients had metastatic disease, and 58 (31.9%) patients had operable breast cancer. Patients were classified into four subtypes according to the HR and HER2 status based on immunohistochemistry. A full assay profile was available for 177 samples of which 84 (44.4%) samples had HR+/HER2-, 17 (9.0%) samples had HR+/HER2+, 13 (6.9%) samples had ER-/HER2+, and 63 (33.3%) samples were TNBC. There were 24 patients with known pathogenic BRCA1 or BRCA2 germline mutation. The detailed characteristics are summarized in Table 2.

Table 2. Characteristics of enrolled patients and samples

Variables	N (%)
Total number of patients	182
Gender	
Female	181 (99.5)
Male	1 (0.5)
Patient age when tissue done NGS	
Median (range)	50 (26-84)
Total number of samples	189
Breast cancer subtypes based on the IHC	
HR+/HER2-	84 (44.4)
HR+/HER2+	17 (9.0)
HR-/HER2+	13 (6.9)
HR-/HER2-	63 (33.3)
Unknown	12 (6.3)
Site of tissue	
Primary breast	106 (56.1)
Metastatic site	83 (43.9)
Lymph node	6 (3.2)
Soft tissue	17 (9.0)
Skin	8 (4.2)
Visceral metastatic site	
Liver	33 (17.5)
Lung	13 (6.9)

Others*	6 (3.2)
Distant metastasis at the time of NGS	
Yes	161 (85.2)
No	28 (14.8)
Number of non-synonymous mutation per sample	
Median (range)	7 (0-22)

NGS, Next-generation sequencing; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; BRCA, Breast cancer gene

*Other site include ovary and salpinx (N=2), bone (N=2), muscle (N=1), and pleural effusion (N=1).

Sequencing results

From October 2016 to March 2020, 340 samples from 255 patients were submitted for NGS, 99 samples using SNUH-FIRST pan-cancer panel v2, 67 samples using v3, and 174 samples using v3.1. Of these 340 samples, 74 failed to process due to insufficient tumor tissue in 57 cases and poor tissue quality in the remaining 17 cases. Altogether, 66 samples were reported to have failed quality control during the PCR process, and 4 samples failed during final sequencing. Two samples in which the tissue sequenced was not that of a breast cancer, and three cases in which the patient had another active malignancy at the time of sampling, were excluded. Finally, 189 samples from 182 patients were analyzed (Figure 1). A total of 1, 428 variants in 243 genes were detected. At least 1 genomic alteration was observed in 99.5% (188/189) of sequenced samples, and the median number of genomic alterations in each sample was 7.

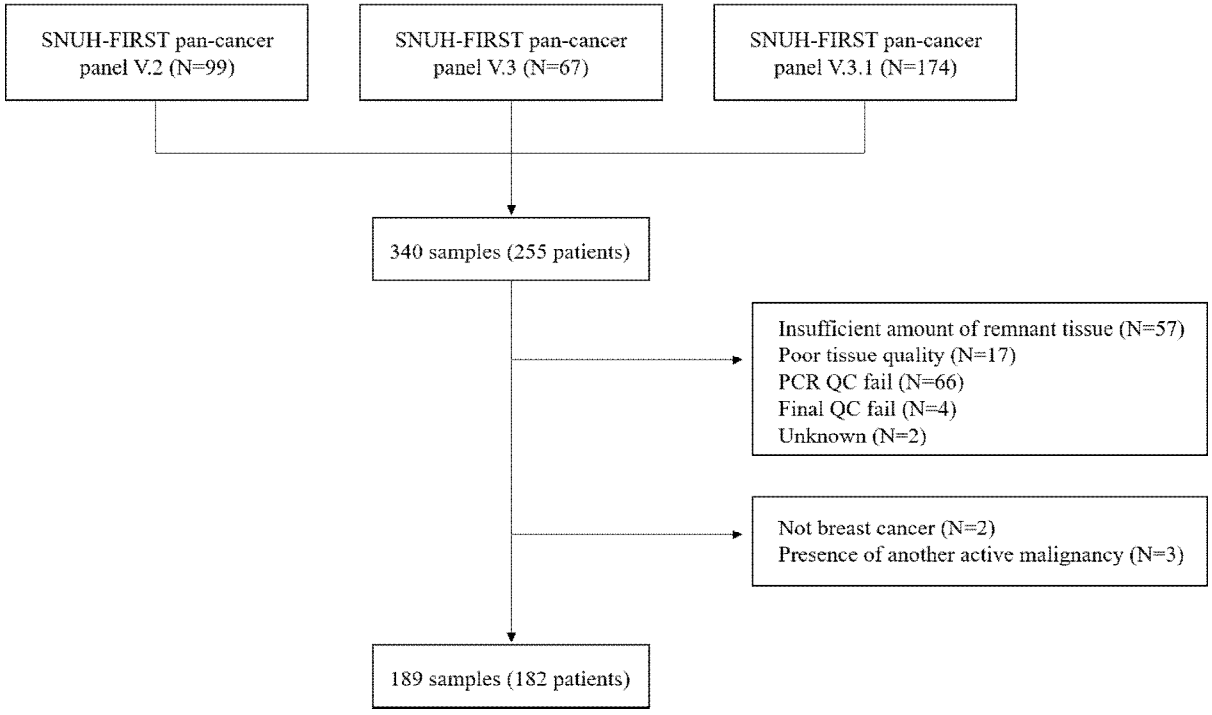


Figure 1 Patient flow of conducting SNUH-FIRST pan-cancer panel with breast cancer

Figure 2 Landscapes of detected variants by subtypes.
Frequently detected genomic alterations are shown. Clinical parameters for each samples are shown in the top panel.

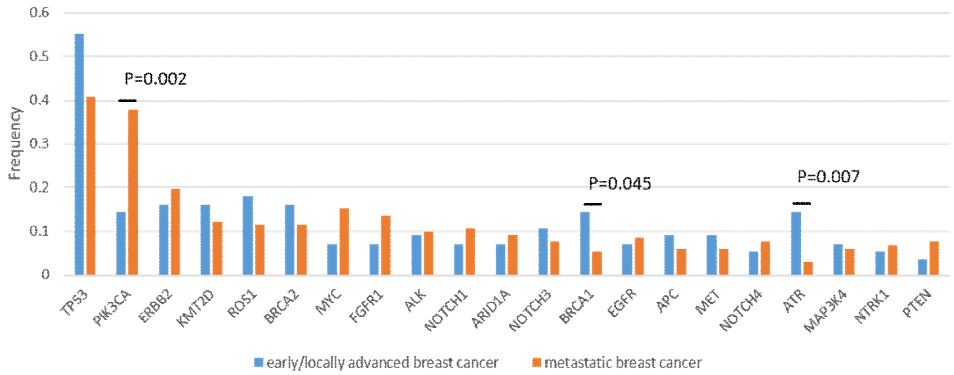


Figure 3 Frequency of genetic alteration between early/locally advance breast cancer and metastatic breast cancer

The frequency of PIK3CA alterations was high in metastatic breast cancer, and the frequency of BRCA1 and ATR were high in early or locally advanced breast cancer

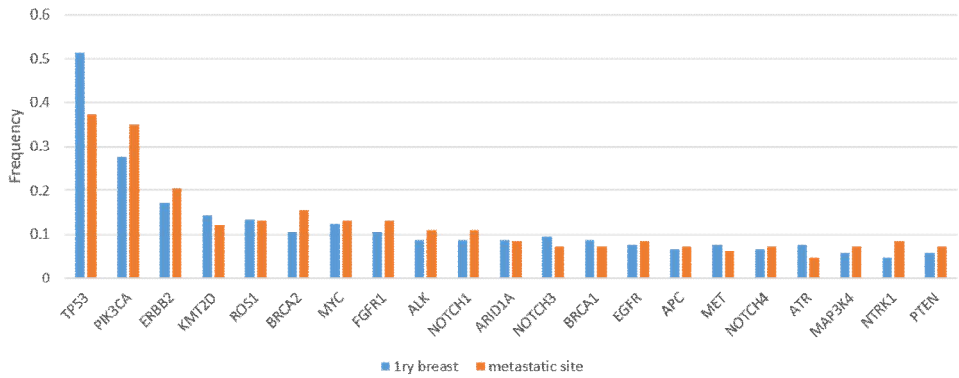


Figure 4 Frequency of genetic alteration between primary breast and metastatic sites

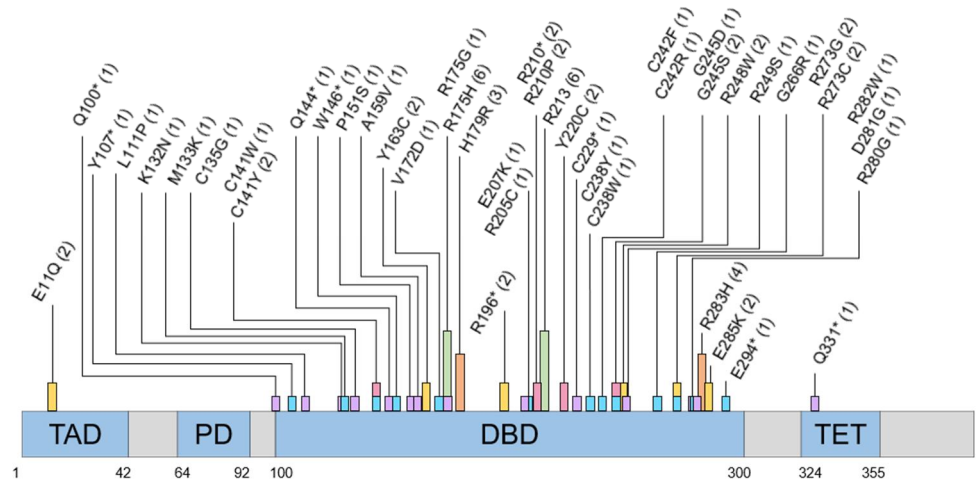
There were no differences in the frequencies of detected genetic alterations in primary breast or metastatic site

When analyzed using only 111 genes included in common in the three versions of the panel, there were 893 SNV, 146 copy number alterations, and 27 copy number deletions. TP53 (59.8%), PIK3CA (31.2%), BRCA2 (19.0%), and ERBB2 (18.5%) were most frequently mutated. Figure 2 shows the frequently ($\geq$3%) identified alterations in all patients. In general, mutation rates were higher in patients with metastatic breast cancer and slightly higher in metastatic site compared to primary breast cancer. In the HR+/HER2- subgroup, PIK3CA (44.6%), TP53 (21.7%), BRCA2 (18.1%) mutation, FGFR1 (9.6%), and Myc (8.4%) amplification were frequently found. In the HER2+ subgroup (ER- or HR+), TP53 (66.7%), PIK3CA (36.7%) mutation, and ERBB2 amplification (80%) were frequently found. In particular, ERBB2 amplification was confirmed in 25 of 30 patients whose sequencing sample was HER2 + (by IHC or FISH) breast cancer. In the TNBC subgroup, TP53 (66.1%), ROS1 (19.4%), KMT2D (17.7%), BRCA1 (14.5%) mutations were frequently found.

There was no significant difference between the overall frequency of alteration by breast cancer stage (early or locally advanced breast cancer vs. metastatic breast cancer, p=0.799) or tissue acquisition site (1ry breast vs. metastatic site, p=0.688). The rate of PIK3CA mutations is high in metastatic breast cancer samples (p=0.004), and the rate of BRCA1 and ATR mutations were significantly high in early or locally advanced breast cancer (p=0.045, 0.007) (Figure 3). There was no significant difference in frequency between tissue obtained from primary breast and metastatic site (Figure 4).

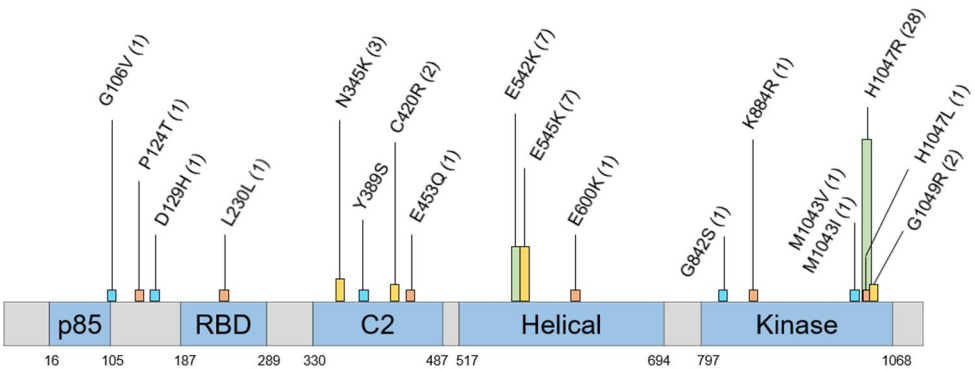
For the genes with frequent mutation, Figure 5 shows the type and frequency of each mutation. TP53 somatic mutations were observed in 121 samples and most of the mutations are located in the DNA binding domain of the protein. PIK3CA somatic mutations observed in 66 samples and mainly located in the calcium/lipid-binding region and kinase domain. BRCA2 mutations observed in 37 samples and most of pathogenic mutations located in RAD51 binding domain and DNA binding domain. ERBB2 somatic mutations were observed in 35 samples, but none of them were the pathogenic kind.

A.



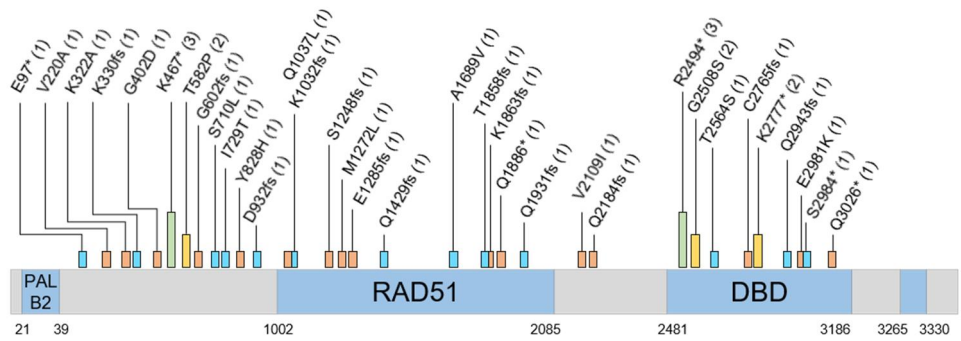
TP53 somatic mutations observed in 121 samples. The mutation discovered are graphed with the amino acid substitution. TAD, transactivation domain; PD, proline-rich domain; DBD, DNA binding domain; TET, tetramerization domain

B.



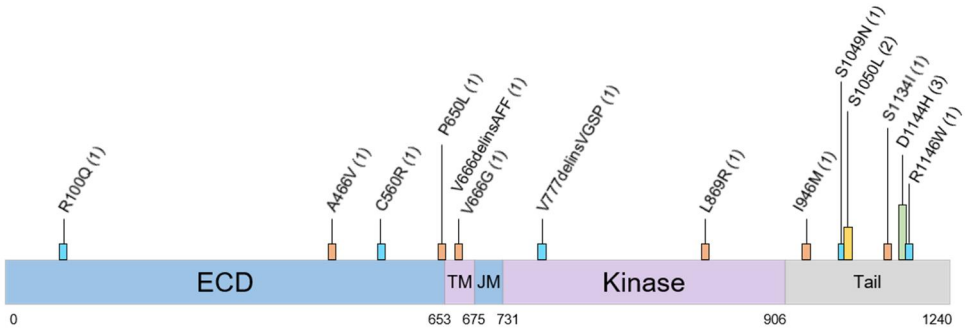
PIK3CA somatic mutations observed in 66 samples. The mutations discovered are graphed with the amino acid substitution. p85, PI3K p85 regulatory subunit binding domain; RBD, Ras binding domain, C2, C2 calcium/lipid-binding region; helical, PI3K accessory (helical) domain; Kinase, PI3/4-kinase domain.

C.



BRCA2 mutations observed in 37 samples. The mutations discovered are graphed with the amino acid substitution. PALB2, PALB2 binding domain; RAD51, RAD51 binding domain, DBD, DNA binding domain

D.



ERBB2 somatic mutations observed in 35 samples. The mutations discovered are graphed with the amino acid substitution. ECD, extracellular domain; TM, transmembrane domain; JM, juxtamembrane domain

Figure 5. The distribution of frequently detected somatic mutation

Two public databases of metastatic breast cancer, "the SAFIR01, SAFIR02, SHIVA, or MOSCATO prospective trials" database sequenced 216 samples of 216 metastatic breast cancer patients, and "the metastatic breast cancer project" database sequenced 237 samples of 180 metastatic breast cancer patients, were compared with sequencing results of 124 metastatic breast cancer patients. There were differences in the genes covered in the two databases and SNUH-FIRST pan-cancer panels. So, among the mutated genes commonly found in the two public datasets, only genes that are included in three versions of our panel are compared. Genes such as CDH1, GATA3, KMT2C, TBX3, RNF213, RELN, NCOR1, PCLO, SPEN, which are not included in our panel were excluded. The frequency of TP53, KMT2D, BRCA2, ATM, and NOTCH3 was significantly high in SNUH-FIRST pan-cancer panel than two public databases. And the frequency of MAP3K1, AKT1, MAP2K4 was significantly high in SNUH-FIRST pan-cancer panel and SAFIR/SHIVA/MOSCATO database than Metastatic breast cancer project database. The frequency of ESR1, NF1, FOXA1, etc. seemed to be low in our results, but not significant. (Figure 6).

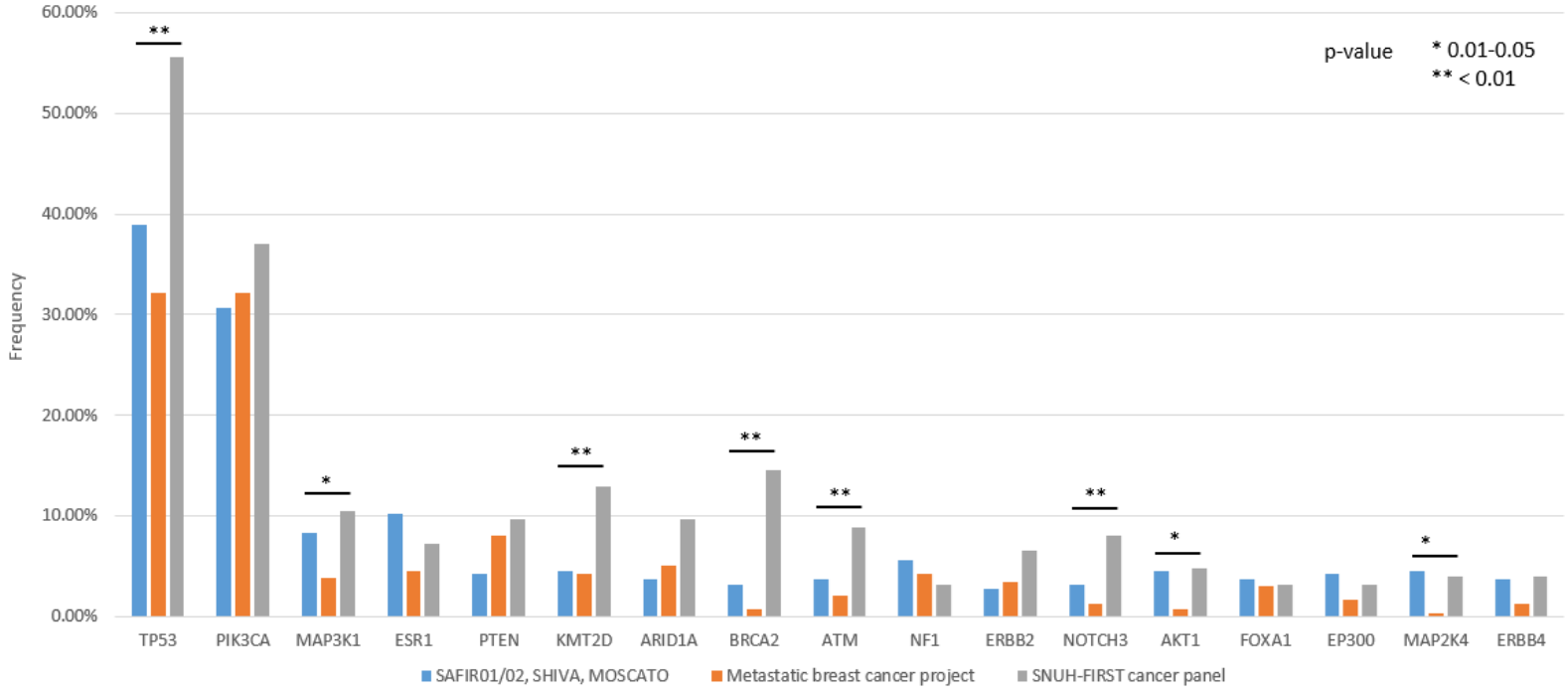


Figure 6 Comparison of frequently detected mutation in metastatic breast cancer

Two public metastatic breast cancer databases were compared with SNUH-FIRST pan-cancer panel results.

Clinical implication

To determine how many of the mutations have clinical significance, the OncoKB precision oncology knowledge database was utilized[52]. Of the 182 patients sequenced, 121 (66.4%) patients had clinically pathogenic alterations according to the OncoKB database (level 1-4), and 112 (61.5%) patients had clinically actionable alterations (OncoKB level 1-3). Among 124 patients with metastatic breast cancer, sequencing results were clinically applicable in 27 (21.8%) patients. 22 patients received approved HER2-targeted therapy, and 5 (4.0%) patients were enrolled into appropriate new targeted agents clinical trial based on sequencing results (2 administrated talazoparib, 3 enrolled in clinical trials) (Figure 7 and Table 3).

None of the 48 patients with PIK3CA pathogenic mutations received PI3K inhibitor trial, because 2 patients had already participated in the PI3K inhibitor trial prior to sequencing, and others did not meet the eligibility criteria for trials. Among 3 patients with ESR1 mutation, 2 patients had already administrated fulvestrant before, and 1 patient had visceral disease and was unsuitable for endocrine treatment

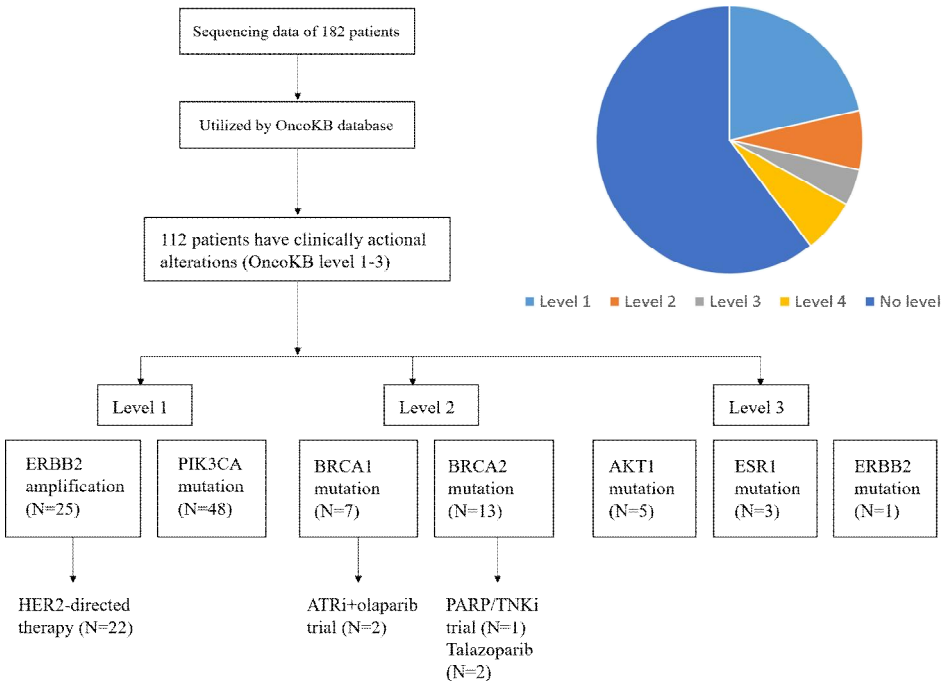


Figure 7 Patient with clinically actionable alteration in OncoKB database

Among 124 patients with metastatic breast cancer, sequencing results were clinically applicable in 27 (21.8%) patients, and 5 (4.0%) patients were enrolled into appropriate new targeted agents clinical trial

Table 3 Detected variants in annotated in OncoKB with targeted therapy

Gene	Protein sequence change	Number of patients	Level	Drugs
AKT1	E17K	5	3A	AZD5363
BRCA1	Y83*	1	2	Olaparib, Talazoparib
	Q333*	1		
	E1163fs	4		
	V1786fs	1		
BRCA2	K467*	2	2	Olaparib, Talazoparib
	G602fs	1		
	Q1886*	1		
	K2777*	2		
	R2494*	2		
	S2984*	1		
	Deletion	2		
	splicing	2		
CDK12	Amplification	8	4	Pembrolizumab, Nivolumab, Cemiplimab
ERBB2	Amplification	25	1	Trastuzumab, Pertuzumab, Margetuximab, Tucatinib, Lapatinib, Neratinib, Poziotinib, Ado-Trastuzumab Emtansine, Trastuzumab-Deruxtecan
	L858R	1	3A	Neratinib
ESR1	Y537S	1	3A	Fulvestrant, AZD9496
	D538G	2		
FGFR1	Amplification	4	4	FGFR inhibitor; AZD4547, Erdafitinib, BGJ398, Debio1347
	N577K	1		
FGFR2	Amplification	2	4	FGFR inhibitor; AZD4547, Erdafitinib, BGJ398, Debio1347
KRAS	Amplification	1	4	Cobimetinib, Binimetinib, Trametinib
	G12V	1		

PIK3CA	G106V	1	1	Alpelisib+ Fulvestrant,
	N345K	2		GDC0077,
	C420R	2		Copanlisib+Fulvestrant
	E542K	5		
	E545K	5		
	M1043I, M1043V	2		
	H1047R	25		
	G1049R	2		
	N345K	2		

In addition to 5 patients who received alteration-matched treatment, 8 patients were enrolled in an alteration-unmatched clinical trial. Table 4 showed detailed genetic alterations and subsequent treatment in these 13 patients. NGS results from two patients who had not previously undergone the germline BRCA test found out germline BRCA mutation using tumor tissue, and subsequently got a chance to receive PARP inhibitor talazoparib. Patient A was previously treated with two lines of endocrine treatment for metastatic breast cancer with ER + breast cancer. The patient did not know the BRCA status because she did not meet the criteria for BRCA testing under Korean national health insurance reimbursement guideline. As the cancer panel revealed that she had a pathogenic BRCA2 germline mutation, she has been currently receiving talazoparib for 4.5 months with partial response (Figure 8A). The other, Patient B was TNBC, to whom two lines of cytotoxic chemotherapy was previously administrated, followed by the administration of talazoparib, maintained for 2 months, and then started on the next anticancer treatment due to disease progression.

Five patients were identified with homologous recombination deficiency (HRD) genes, such as BRCA1/2, ATM, and CHEK2, and enrolled into the clinical trials administrating a poly(ADP-ribose) polymerase (PARP)/Tankyrase (TNK) dual inhibitor. In addition, seven patients were thought to have a mutation in the DNA damage response (DDR) or related gene; these were enrolled in a clinical trial administrating Ataxia telangiectasia and Rad3-related (ATR) inhibitors or ataxia-telangiectasia mutated (ATM) inhibitors.

Table 4 Genomic profiles of patient who had treated based on the results sequencing

	Gene	AA change	AF (%)	Clinical significance	No. of previous chemotherapy*	Treatment	Best response	PFS (months)
Patient A	BRCA2	R2494*	89.6	Pathogenic	0**	Talazoparib	PR	5.5
Patient B	BRCA1	splicing	94	Pathogenic	2	Talazoparib	PD	2
Patient C	BRCA2	splicing	38.6	Pathogenic	2	PARP/TNKi ***	PD	2
Patient D	PALB2	E1018D	39		2	PARP/TNKi	SD	4
	RAD51C	G3W	16					
Patient E	ATR	M2087V	59		2	PARP/TNKi	PD	1
Patient F	ATM	L2258fs	45		5	PARP/TNKi	SD	1
Patient G	PTEN	D326fs	54		4	PARP/TNKi	SD	4
Patient H	PIK3CA	H1047R	54	Pathogenic	1	Olaparib+ATRi	PD	3
Patient I	PIK3CA	H1047R	22	Pathogenic	1	Olaparib+ATRi	SD	5.5
Patient J	BRCA1	E1163fs	38	Pathogenic	1	Olaparib+ATRi	SD	24
Patient K	BRCA1	Y83*	53	Pathogenic	1	Olaparib+ATRi	PR	14
Patient L	FANCG	Q356*	50	Pathogenic	3	Olaparib+ATMi	SD	7

	FANCA	W1063*	37	Pathogenic				
Patient M	AKT1	E17K	40	Pathogenic	5	Olaparib+ATMi	SD	7.5

NGS, next-generation sequencing; AA, amino acid; AF, allele frequency; PFS, progression free survival

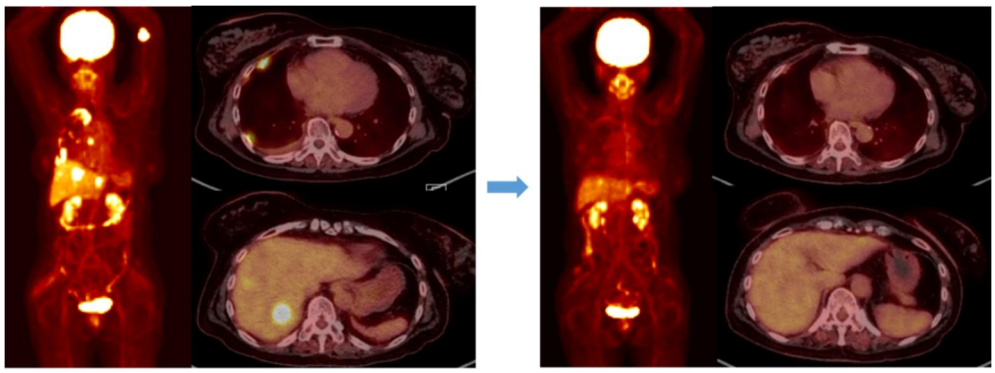
* The number of cytotoxic chemotherapy performed for metastatic breast cancer was included.

** This patient had 2 lines of endocrine therapy previously.

*** It is clinical trial consist of a poly(ADP-ribose) polymerase (*PARP*)/Tankyrase (*TNK*) dual inhibitor

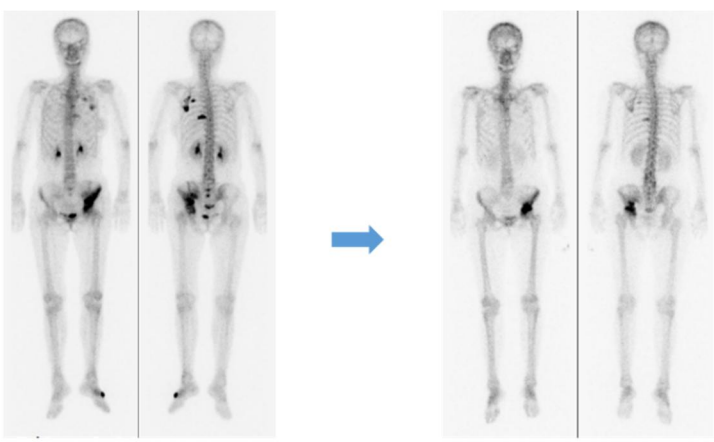
Patient J was administered palliative gemcitabine and paclitaxel for metastatic breast cancer; however, it was discontinued because of intolerance. Subsequently, the patient participated in a clinical trial to administer an ATR inhibitor, and the PARP inhibitor olaparib. Since then, for two years, the patient has been taking the drug well, maintaining a stable disease, and is much more tolerable to treatment than with the previous cytotoxic chemotherapy (Figure 8B).

Patient K also received paclitaxel and carboplatin treatment for metastatic breast cancer and achieved near-complete remission. However, she reached a point where she could not sustain chemotherapy due to severe neurotoxicity. The patient was enrolled in a clinical trial administering ATR inhibitors and PARP inhibitors, based on the results of the cancer panel. The patient had brain metastasis from the beginning, and after 14 months, disease progression was confirmed only through brain lesions. However, the patient maintained a near-complete remission status systemically. Thus, she continued to administer the trial drugs for 26 months while controlling her brain disease with gamma knife surgery (Figure 8C).



Pretreatment PET (2019.9)　　Posttreatment PET (2020.5)

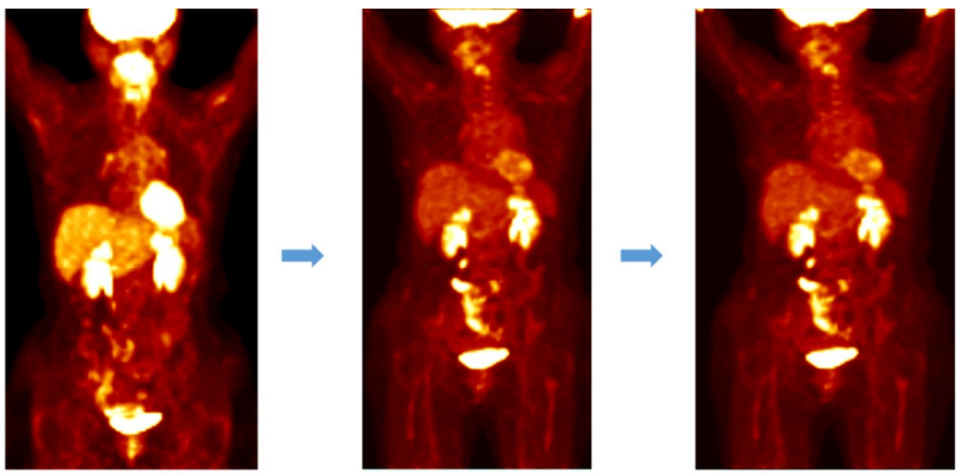
A. A 64-year-old female (patient A) who experienced disease progression after two lines of endocrine treatment for metastatic breast cancer, started talazoparib after cancer panel detect germline BRCA2 mutation and achieved a partial response with a significant reduction in SUV on PET scan (Maximal SUV of right pleural lesion, 9.6 to 2.9, and maximal SUV of liver mass, 15.4 to 4.3, respectively). PET, positron emission tomography; SUV, standard uptake value



Pretreatment bone scan (2018.2)　　Posttreatment bone scan (2020.4)

B. A 49-year-old female (patient J) discontinued 1st line chemotherapy due to side effects and participated in a clinical trial to apply ATR inhibitor and PARP inhibitor. At the time of

enrollment, the patient only had bone metastasis, and it was improved with the ATR and PARP inhibitors. ATR, Ataxia telangiectasia and Rad3-related; PARP, poly(ADP-ribose) polymerase



C. A 57-year-old female (patient K) achieved near-complete remission after 1st line cytotoxic chemotherapy but discontinued because of neurotoxicity. The patient enrolled in a clinical trial with ATR inhibitors and PARP inhibitors, and maintained near-complete remission status systemically, except for brain metastasis controlled by gamma knife surgery. ATR, Ataxia telangiectasia and Rad3-related; PARP, poly(ADP-ribose) polymerase.

Figure 8 Examples of NGS profiling leading patients to clinical trials

Discussion

For the purpose of obtaining diagnostic assistance and linking patients with specific mutations to appropriate clinical trials, our center established a targeted sequencing based NGS test SNUH-FIRST pan-cancer panel. In this study, utility of the SNUH-FIRST pan-cancer panel in patients with breast cancer was explored and its clinical significance was also investigated.

In the 189 samples and 182 patients sequenced, 1,428 non-synonymous mutations were detected. In general, mutation rates were higher in patients with metastatic breast cancer and slightly higher in metastatic site as compared to primary breast cancer. Overall PIK3CA, TP53 mutations and ERBB2 amplification were frequently found. In HR+ breast cancer samples, PIK3CA mutation and FGFR1 and MYC amplifications were especially high. In most of the HER2+ breast cancer samples, ERBB2 amplification was found. Some of the HER2+ breast cancer samples had ERBB2 mutations but none of them were the pathogenic type.

As compared to previously reported metastatic breast cancer database, the overall frequency of mutation was high in our test because SNUH-FIRST pan-cancer panel contained a relatively small number of genes, with the intention of identifying only clinically meaningful or targetable genes. Not all patients who were sequenced in the SNUH-FIRST pan-cancer panel for breast cancer had metastatic disease. Instead, by allowing physicians to conduct examinations freely during the treatment process, more patients, who were refractory to treatment or were considered to have high mutational burden were included. Owing to this limitation in enrollment, it cannot be judged whether the difference from other metastatic breast cancer database is a

characteristic of Korean breast cancer patients. However, the frequency of TP53, BRCA2, and ATM mutations was especially high in our results. TP53 mutation is known to be different between the Asian and Caucasian population. Zhang et al. reported that TP53 mutation was significantly higher in Chinese cohort than in TCGA database[53].

Out of 124 metastatic breast cancers, sequencing results were clinically applicable in 27 (21.8%) patients, and 5 (4.0%) patients were able to receive genetic alteration-matched treatment. Moreover, another 8 patients were enrolled in an alteration-unmatched clinical trial by referring to sequencing results. In the NCI-MATCH trial that assigned patients according to DNA targeted sequencing, assign rate was less than 2% for each sub-protocol[54]. Moreover, in another TGS-based trial, the SAFIR trial, assign rate of the genotype-matched trial was 5%[55]. Considering that only a small number of patients could be assigned to proper treatment in this sequencing-based trial, it can be said that 4% is a fairly high rate, although all possible treatment options were included in our case.

In this study, pathogenic mutation of germline BRCA was detected through target sequencing in 15 patients. In particular, two patients who had not tested for germline BRCA using peripheral mononuclear cells, were found to have germline BRCA mutation in SNUH-FIRST pan-cancer panel target sequencing. One patient started administrating talazoparib and another was enrolled into the clinical trial. In Korea, due to the issue of insurance standards, there are restrictions on BRCA inspection. Considering high prevalence of germline BRCA[56], and that there is an effective treatment when BRCA is identified, it is a significant pitfall that germline BRCA

tests cannot be performed. In patients who do not meet insurance standards, but have a high probability of BRCA mutation, such as an old patient with TNBC, this obstacle to BRCA test is a severe problem. As the NGS test is partly covered by insurance, the patient is tested with less financial burden if the germline BRCA is detected by target sequencing. Further research therefore, is needed in this direction.

The limitations of this study are as follows: As the SNUH-FIRST pan-cancer panel of the present application used a sequencing method that was developed during the research period, the included gene or sequencing success rate changed continuously. This may have caused a difference depending on the panel version used in the analysis. In addition, considering the low test success rate in the case of old tissue, tissue biopsy of a metastatic site, or insufficient amount, or a large discrepancy in the sample used may also compromise the validation of the study. Besides, since all patients sequenced with breast cancer tissue were included in the analysis, many factors could lead to variations in the sequencing results, such as disease status and history of other carcinomas. Due to the heterogeneity in this patient group, it was not possible to perform additional analysis, such as determining the relationship between detected mutations and prognosis.

In conclusion, through the SNUH-FIRST pan-cancer panel, the mutational landscape of breast cancer patients in a single center was elucidated and practical value in the treatment of real patients with breast cancer was identified. However, there is a patient bias included in this study; therefore, to really observe the characteristics in Korean breast cancer patients, it is necessary to validate the findings in a larger number of patients.

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국문초록

서론: 차세대 염기서열분석법 (Next Generation Sequencing) 은 기존의 염기서열분석법에 비하여 빠르게 많은 변이를 분석할 수 있는 방법이다. NGS 검사를 통해 질병과 관련된 변이를 알게 되고 이에 대한 치료제를 탐색하게 되면서, 암 치료에 많은 발전이 있었다. 본 논문에서는 한국 유방암 환자들에서의 흔한 변이를 확인하고 이러한 정보가 실제 환자의 치료에 어떻게 사용되는지 분석 하였다.

방법: 본 연구에서는 서울대병원에서 유방암 조직으로 NGS 검사를 시행한 환자의 병리 결과, NGS 결과 및 의무기록을 후향적으로 조사하였다. NGS 결과에서 흔한 변이를 관찰하고 기술하였고, 실제 환자에서 적용된 예시를 살펴 보았다.

결과: 2016년 10월부터 2020년 3월까지 NGS 검사를 시행한 유방암 환자 중 182명의 sample 189개에서 분석을 시행하였다. 총 243개 gene 에서 1,428가지 variant 가 확인되었고, 샘플당 변이 수의 중앙값은 7(0~22)개였다. 전체 샘플에서 가장 흔한 변이는 TP53 (59.8%), PIK3CA (31.2%) 등에서 발견되었고, subtype 에 따라서는 HER2 양성에서 ERBB2 amplification 이 흔하고 (80%), 삼중음성유방암 (triple-negative breast cancer) 에서 TP53 (66.1%), BRCA1 (14.5%) 외에 ROS1 (19.4%), KMT2D (17.7%) 로 높은 빈도로 확인되었다. 61.5% 의 환자에서 임상적 유용성이 있는 변이가 발견되었고, 22% 의 환자에서 이를 임상적으로 적용 가능했으며, 특히 4% 의 환자는 이 결과를 토대로 적절한 표적 치료를 시행하는 임상 시험 등에 연결되었으며, 일부 환자에서 매우 효과적인 결과를 보여주었다.

결론: 본 연구는 서울대병원 단일 기관에서 유방암 환자의 NGS 경험 및

이 결과를 환자의 치료로 연결할 수 있음을 보여주었다. 이를 통해 NGS 결과가 환자의 실제 치료에 도움이 되며, 특히 우리나라 보험 환경에서 유용함을 확인할 수 있었다.

주요어 : 유방암, 차세대염기서열분석, 유전 변이
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