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Predictive Role of TP53, PIK3CA and MLL2 in ER+ HER2+ Breast Bancer: Biomarker Analysis of Neo-ALL-IN [NCT 01275859]

JI HYUN PARK[1,2], JIN-HEE AHN[1], JEONG EUN KIM[1], KYUNG HAE JUNG[1], GYUNGYUB GONG[3], HEE JIN LEE[3], BYUNG-HO SON[4], SEI-HYUN AHN[4], HAK-HEE KIM[5], HEE JUNG SHIN[5], DAE-HYUK MOON[6], DEOKHOON KIM[3] and SUNG-BAE KIM[1]

[1]*Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea;*
[2]*Department of Hemato-Oncology, Konkuk University Medical Center, Univeristy of Konkuk College of Medicine, Seoul, Republic of Korea;*
[3]*Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea;*
[4]*Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea;*
[5]*Department of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea;*
[6]*Department of Nuclear Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea*

Abstract. *Background/Aim: Somatic mutations were investigated in 21 patients with postmenopausal estrogen receptor (ER)-positive and human epidermal growth factor receptor-2 (HER-2)-positive (ER+HER2+) breast cancer (BC) treated with neoadjuvant letrozole and lapatinib, to identify their distinct molecular landscape. Patients and Methods: We used tissue samples of 21 patients from phase II Neo ALL-IN cohort, and somatic alterations were examined using targeted exome sequencing performed in Foundation Medicine, Inc. (FMI). Results: TP53 (61.9%) and PIK3CA (57.1%) were the two most frequently mutated genes that were inter-correlated (p=0.026). They were associated with unfavorable clinical outcomes, particularly when accompanying PIK3CA mutations at exon 9 in helical domains. Meanwhile, MLL2 alteration was negatively associated with mutations of TP53 or PIK3CA, and it tended to be present in patients with low KI-67 levels and no initial nodal involvement. Moreover, patients with MLL2 mutations numerically showed more favorable overall response rates (ORR) (80% vs. 56.2%) and better 5-year event-free survival (EFS) rates (100% vs. 87.5%) compared to the wild-type. Conclusion: Mutations in TP53 and PIK3CA hotspot at exon 9 may be potential negative predictors of ER+HER2+ BC treated with neoadjuvant letrozole and lapatinib, while MLL2 inactivating mutation might confer therapeutic benefit in these patients.*

Correspondence to: Deokhoon Kim, PhD, Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea. Tel: +82 230104122, Fax: +82 230108772, e-mail: coonya@gmail.com and Sung-Bae Kim, MD, Ph.D., Department of Oncology, Asan Medical Center, University of Ulsan College of Medicine, 88 Olympic-ro 43-gil, Songpa-gu, Seoul 05505, Republic of Korea. Tel: +82 230103217, Fax: +82 230108772, e-mail: sbkim3@amc.seoul.kr

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Estrogen receptor (ER)-positive and human epidermal growth factor receptor-2 (HER-2)-positive (ER+HER2+) breast cancer (BC), called 'triple-positive' BC, intrinsically harbors biological heterogeneity, but its molecular landscape has not been studied much thus far. As it has been historically regarded as hormone receptor-positive subset of HER2+ BC, patients with ER+HER2+ BC uniformly receive combination chemotherapy with the backbone of anti-HER2 therapy in current standard practice (1). However, because they carry two canonical targets, ER and HER2, that are independently powerful but also intricately interacting (2, 3), prediction of their biologic phenotype and treatment response is occasionally challenging.

In neoadjuvant lapatinib and letrozole in patients with ER+ HER2+ (Neo-ALL IN) (4), we demonstrated feasibility of chemo-free neoadjuvant combination regimen of letrozole

and lapatinib in a small group of Korean patients having postmenopausal ER+HER2+ BC. Although expressional change of immunohistochemical (IHC) ER, Fluorine-18 Fluoroestradiol (^{18}F-FES) positron emission tomography (PET), and tumor-infiltrating lymphocytes (TILs) were suggested as potential clinicopathologic predictors of clinical response in that study, several conflicting issues still remained. The predictive relevance of ER expression and FES-PET highlighted a consistently powerful impact of ER itself to this subtype of BC. Moreover, observed negative association of TILs with clinical outcomes further illuminated substantial influence of luminal biology to ER+HER2+ BC (5-8). These findings collectively suggest a distinct biology of ER+HER2+ BC from typical HER2+ BC (9-11). Therefore, we performed a genomic biomarker study of 21 patients with ER+HER2+ BC from the Neo-ALL In cohort, and attempted to elucidate its biologic complexity by unravelling molecular profiles.

Patients and Methods

Study design and patients. Neo-ALL IN (4) was a single-center phase II study investigating the efficacy and toxicity of neoadjuvant chemo-free combination therapy with letrozole and lapatinib in postmenopausal ER+HER2+ BC. A total of 24 patients received daily oral doses of 2.5 mg letrozole and 1,500 mg lapatinib every 3 weeks for 18 to 21 weeks before surgery. Primary efficacy endpoint was pCR defined as the absence of residual invasive breast carcinoma both in breast and axillary lymph nodes (ypT0/isN0). Clinical overall response rate (ORR), event-free survival (EFS) and overall survival (OS) were secondary efficacy endpoints. Investigation of predictive biomarkers was another secondary exploratory endpoint, and current study presents the first report of genomic analysis performed in the biomarker population of Neo-ALL IN. It was conducted in full accordance with the guidelines for Good Clinical Practice and the Declaration of Hensinki. The study protocol was reviewed and approved by the Institutional Review Board (IRB approval number: No 2007-0729) of Asan Medical Center, and informed consents were achieved from all individual participants included in the study.

Targeted exome sequencing. According to the protocol of the study, collection of baseline tissues from trucut biopsies and serum was mandatory before the initiation of neoadjuvant treatment (4). Tumor samples were archived as formalin-fixed, paraffin-embedded (FFPE) tissues. Somatic alterations were examined using the targeted gene panel of Foundation Medicine, Inc. (FMI) (Cambridge, MA, USA) (12). Specifically, genomic DNA (gDNA) was extracted from 40 μm of unstained FFPE sections, typically 4×10 μm sections, by digestion in a proteinase K buffer for 12-24 h followed by purification with the Promega Maxwell 16 Tissue LEV DNA kit. The extracted gDNA was quantified by a Picogreen fluorescence assay (Invitrogen, Carlsbad, CA, USA). A total of 50-200 ng of gDNA in 50-100 μl water in microTUBEs was fragmented to ~200 bp by sonication (3 min, 10% duty, intensity=5, 200 cycles/burst; Covaris E210; Covaris Inc. Woburn, MA, USA) before purification using a 1.8× volume of AMPure XP Beads (Agencourt, Beverly, MA, USA). SPRI purification and subsequent library construction with the NEBNext kits (E6040S, NEB), containing mixes for end repair, dA addition and ligation, were performed in 96-well plates (Eppendorf, Hamburg, Germany) on a Bravo Benchbot (Agilent, Sanaclara, CA, USA). Indexed (6-bp barcodes) sequencing libraries are PCR amplified with HiFi (Kapa Biosystems, Woburn, MA, USA) for 10 cycles, 1.8× SPRI purified and quantified by qPCR (Kapa SYBR Fast) and sized on a LabChip GX (Caliper). PCR yield was maximized by ensuring that no SPRI beads were transferred to the PCR tube. The baits targeted ~1.5 Mb of the human genome including 4,557 exons of 287 cancer-related genes, 47 introns of 19 genes frequently re-arranged in cancer, plus 3,549 polymorphisms located throughout the genome. The PCR master mix was added to directly amplify (12 cycles) the captured library. After amplification, the samples are 1.8× SPRI purified, quantified by qPCR (Kapa Biosystems, Woburn, MA, USA) and sized on a LabChip GX (Caliper). Libraries were normalized to 1.05 nM and pooled such that each Illumina HiSeq 2000 lane has up to four samples each (32 per flowcell). Sequence data were analyzed using FMI pipeline.

Endpoints and statistical analysis. Descriptive statistics were used to analyze patient characteristics. To find out the clinical significance of genetic aberrations including SNVs and CNVs, the status of mutations were analyzed in association with efficacy outcomes such as ORR, 5-year EFS and OS rates. ORR was defined as the sum of patients with complete response or partial response according to the RECIST v1.1 (13). EFS was defined as the time duration from the initiation date of chemotherapy to first confirmed objective tumor relapse after curative surgery, progression during the neoadjuvant treatment, or death from any cause. OS was defined as survival free of death from any cause. Pearson's Chi-square or Fisher's exact tests were used to evaluate the difference of ORR by mutations. Survival analyses were performed using the Kaplan–Meier (KM) method with log-rank test. All *p*-values were two-sided and statistical significance was accepted at the $p<0.05$ level. PASW (version 20.0; IBM Co., Armonk, USA) was used for all statistical analyses in current study.

Results

Patients. The characteristics of 21 patients finally analyzed are summarized in Table I. The median Allred score of ER was 8 that 71.4% of patients showed strong ER positivity (score 7 or 8). Progesterone (PR) positivity was observed in 12 (57.1%) patients. The median value of Ki-67 index was 30%. During the median follow-up of 54 months (range=49-59 months), 5-year EFS and OS rates were 90.5% and 95.0%, respectively. There has been one additional death since our first report of Neo-ALL IN study, in a patient who experienced rapid progression immediately after completion of neoadjuvant treatment.

Genomic landscape of ER+HER2+ BC. We performed targeted exome sequencing of 21 tumor samples with a median depth of 426x in the targeted region (range=171x~591x). A total of 268 somatic mutations were identified: 215 missense mutations; 14 nonsense mutations; 1 nonstop mutation; 7 splice-site mutations; 1 start-codon

Table I. *Patient characteristics (N=21).*

Characteristics (n, %)	Total
Age, median (range)	57 (49-74)
ECOG performance status	
0	20 (95.2)
1	1 (4.8)
Primary tumor size (cm)	
Median (range)	4 (1.9-8.7)
Clinical stage	
II	7 (39.2)
III	17 (70.8)
Initial nodal involvement	
No	3 (14.3)
Yes	18 (85.7)
Histologic grade	
Well or moderately differentiated	17 (81.0)
Poorly differentiated	4 (19.0)
Ki-67 index of initial tissue	
Median (range)	30 (10-80)
Tumor infiltrating lymphocyte (TIL) (%)	
Median (range)	10 (0-90)
Strong ER expression	
Allred score 7-8	15 (71.4)
PR positivity	12 (57.1)
No. of treatment cycles, median (range)	7 (2-8)
Type of surgery	
MRM	12 (57.1)
BCO	8 (38.1)
Others	1 (4.8)
Adjuvant therapy	
Cytotoxic chemotherapy	21 (100)
Trastuzumab and hormonal therapy	20 (95.2)
Radiotherapy	16 (76.2)

ECOG: Eastern Cooperative Oncology Group; ER: estrogen receptor; PR: progesterone receptor; MRM: modified radical mastectomy; BCO: breast conserving operation.

mutation; and 30 small insertions/deletions (indels). The median number of somatic mutations per sample was 12.8 (rage=0-24). C>T transitions were predominant as previously reported in other studies (Figure 1A) (14).

Frequent somatic mutations, and their clinical relevance. We identified a total of 145 genes harboring protein-altering somatic mutations, and 25 of them were recurrently mutated in at least three patients (Figure 1B). Frequent alterations in the cohort are summarized in Table II. In addition, Figure 1B schematically depicts frequently mutated genes in association with their key pathologic features. *TP53* and *PIK3CA* were the two most common genes harboring somatic mutations in the cohort, followed by *BRCA2*, *FAT1*, *MLL2*, and *SPTA1* (Table II).

TP53 mutation was the most prevalent somatic mutation (13/21, 61.9%). Fourteen somatic mutations in *TP53* were overlapped with the COSMIC database and twenty somatic mutations in *TP53* were located in P53 DNA-binding domain and the p53 tetramerization motif (Figure 2A). As expected, somatic mutations in *TP53* was more frequently observed in patients with an HER2 IHC score 3 than in 2 (70.6% *vs*. 25.0%, p=0.091).

PIK3CA was a second most commonly mutated gene in this cohort (12/21, 57.1%). Eight cases of *PIK3CA* mutations were found in hotspot with a slight preference of H1047 alteration at exon 20 in kinase domain, which was significantly more frequent compared with TCGA (38% *vs*. 14%) (Figure 2B, C). Unlike *TP53* somatic mutation, there was no significant association of *PIK3CA* mutation with either HER2 or ER Allred score.

Somatic mutations in *TP53* and *PIK3CA* were positively associated (p=0.026), and patients with either *TP53* or *PIK3CA* mutations tended to show worse ORR and 5-year EFS rates (84.6% *vs*. 100%, p=0.26, 85.7% *vs*. 100%, p=0.31) although it did not reach statistical significance (Table III, Figure 3A and B). Interestingly, progression or death observed in the cohort was found in two patients who simultaneously harbored *TP53* and *PIK3CA* somatic mutations.

MLL2, a potential epigenetic regulator, and its clinical impact. All somatic mutations in *MLL2* were protein-altering variations (missense mutations, in-frameshift deletions). Somatic mutations of *MLL2* showed a positive association with relatively low HER2 expression that MLL2 mutation was observed significantly more in patients with HER2 IHC score 2 than with score 3 (75.0% *vs*. 11.8%, p=0.008). Also, the presence of *MLL2* mutation was negatively associated with somatic mutations in *TP53* (p=0.027) or *PIK3CA* (p=0.147). Interestingly, it was more frequently observed in patients with TILs <10%, low KI-67 level, and the absence of initial nodal involvement (data not shown), although it did not meet statistical significance. Of note, patients with MLL2 mutation showed more favorable ORR compared to the wild-type (80% *vs*. 56.2%, p=0.340) (Table III), and presented better 5-year EFS rates (100% *vs*. 87.5%, p=0.422) (Figure 3C). Even among 14 patients with PIK3CA mutations, patients with MLL2 mutations showed a better ORR (100% *vs*. 50%, p=0.186) and EFS compared to patients without mutations (100% *vs*. 83.3%, p=0.555).

Frequently identified copy number alterations. Among the recurrent copy number alterations including *ERBB2*, *MYC*, *RUNX1T1* and *SPOP*, *ERBB2* was the most frequently amplified gene in this cohort (17/21, 81%), and approximately 80% of patients showed a strong HER2 expression with IHC score 3. As expected, HER2 IHC score and ERBB2 amplification showed a significant positive association (p=0.002) that 75% of patients (3/4) who showed low HER2 expression (IHC score 2) did not present *ERBB2* copy number amplification.

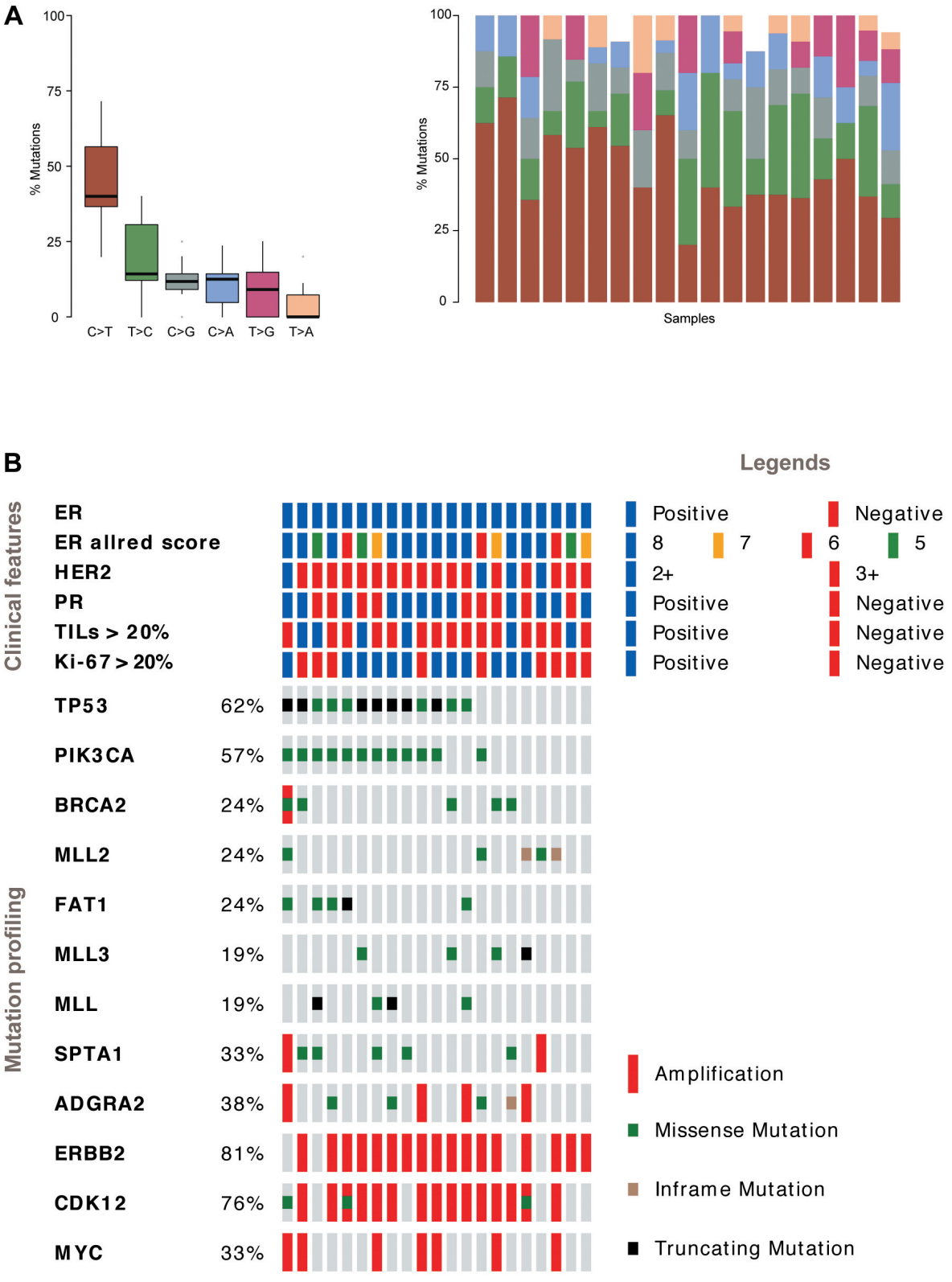


Figure 1. *Plot of frequencies and patterns of recurrently altered genes among patients in association with key pathologic features of ER+HER2+ BC. (A) Overall distribution of six different conversions and a stacked barplot of conversions in each sample. (B) Oncoplot for recurrently altered genes in 21 ER+HER2+ BCs.*

Table II. *Frequently identified genetic alterations in the cohort (N=21).*

SNV/INDEL			CNV			
Gene	Patients (No.)	Frequency (%)	Gene	Type	Patients (No.)	Frequency (%)
TP53	13	61.9%	CDK12	AMP	15	71.4%
PIK3CA	12	57.1%	ERBB2	AMP	17	81%
FAT1	5	23.8%	MYC	AMP	7	33.3%
MLL2	5	23.8%	NBN	AMP	6	28.6%
BRCA2	5	23.8%	RUNX1T1	AMP	6	28.6%
SPTA1	5	23.8%	SPOP	AMP	6	28.6%
MLL3	4	19%				
MLL1	4	19%				
ADGRA2	4	19%				

SNV: Single nucleotide variant; INDEL: insertion and deletion of a base; CNV: copy number variant; AMP: amplification.

In the study, *RUNX1T1* and *MYC* amplifications showed a tendency of co-occurrence (p=0.002) that 5 of 6 patients with *RUNX1T1* amplification concurrently harbored *MYC* amplification (Figure 1). *MYC* amplified patients (n=7) consistently showed strong ER positivity by IHC. Among 6 patients with *RUNX1T1* amplification, 2 patients also presented *RUNX1* amplification. Interestingly, patients with *RUNX1T1* or MYC amplification showed numerically better ORR (83.3% *vs*. 53.3%, p=0.201, 100% *vs*. 57.9%, p=0.243, 85.7% *vs*. 50.0%, p=0.112) (Table III), and patients with *RUNX1T1* amplification also demonstrated better EFS outcomes than patients without amplicifation (100% *vs*. 86.7%, p=0.363). However, *MYC*-amplified patients showed poorer 5-year EFS rate compared with non-amplified patients (85.7% *vs*. 92.9%, p=0.577) (data not shown).

Exploratory novel CDK12-IKZF3 rearrangement. We found a novel CDK12-IKZF3 rearrangement in one patient (Figure 4). This rearrangement has been reported in TCGA bladder urothelial carcinoma only. However, the effect of the specific rearrangement in tumor biology has not been explored yet. Edgren *et al*. reported that VAPBIKZF3 fusion identified in RNA-seq may important for growth and survival of breast tumor (15).

Molecular profiles of patients who experienced progression or death.We focused on the genomic landscape of 2 patients who experienced rapid progression or death during or shortly after the neoadjuvant treatment. One patient progressed during the neoadjuvant treatment and prompted to early mastectomy followed by adjuvant treatment, and another showed rapid progression immediately after completing neoadjuvant treatment (4). The latter demonstrated early distant relapse after mastectomy, which finally led her to death regardless of vigorous palliative chemotherapies.

Table III. *Predictive value of frequently identified genetic alterations with clinical responses.*

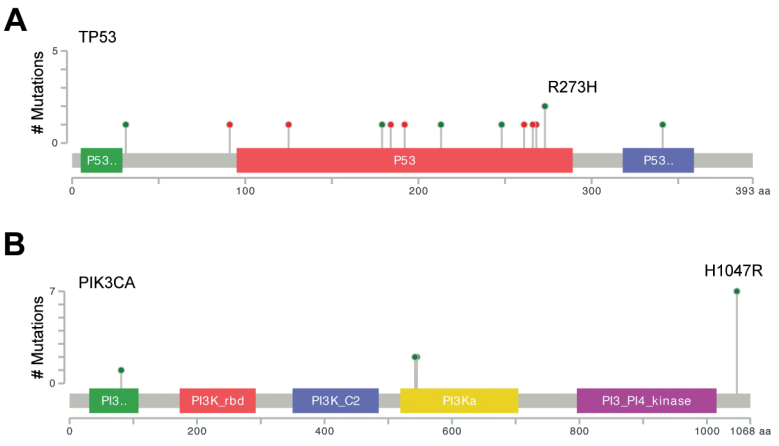
SNVs		Responsive	Non-responsive	p-Value
TP53	Wild, N (%)	6 (75.0)	2 (25.0)	0.332
	Mutant, N (%)	7 (53.8)	6 (46.2)	
PIK3CA	Wild, N (%)	5 (71.4)	2 (28.6)	0.525
	Mutant, N (%)	6 (50.0)	6 (50.0)	
MLL2	Wild, N (%)	9 (56.2)	7 (43.8)	0.340
	Mutant, N (%)	4 (80.0)	1 (20.0)	
CNVs		**Responsive**	**Non-responsive**	**p-Value**
ERBB2	Not amplified, N (%)	2 (50.0)	2 (50.0)	0.586
	Amplified, N (%)	11 (64.7)	6 (35.3)	
RUNX1T1	Not amplified, N (%)	8 (53.3)	7 (46.7)	0.201
	Amplified, N (%)	5 (83.3)	1 (16.7)	
MYC	Not amplified, N (%)	7 (50.0)	7 (50.0)	0.112
	Amplified, N (%)	6 (85.7)	1 (14.3)	

SNV: Single nucleotide variant; CNV: copy number variant.

Surprisingly, these 2 patients presented a great similarity of SNVs that they simultaneously had *TP53* and *PIK3CA* somatic mutations but did not harbor *MLL2* somatic mutations. Meanwhile, they did not present consistent profiles of CNVs. While one patient harbored abundant copy number amplifications of *KRAS*, *ARFRP1*, *AURKA*, *GNAS*, *PIK3C2G*, *ROS1*, *WISP3*, *ZNF217*, *MYC*, *ERBB2*, and *CDK12*, the other who ultimately died did not bring any type of CNVs.

Discussion

We attempted to investigate a distinct genomic landscape of 21 postmenopausal women with ER+HER2+ BC treated



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Exon	Domain	A.A. Position	A.A. Change	NeoAll-IN	TCGA * non classified
9	Helical domain	E542	E542K	5%	4%
		E545	E545K	14%	7%
			E545Q		
			E545G		
			E545V		
		Q546	Q546K		<1%
			Q546E		
			Q546P		<1%
			Q546R		1%
			Q546L		
		D549	D549N		
20	Kinase domain	H1047	H1047R	38%	14%
			H1047L		1%

Figure 2. *Mutational profile of TP53 and PIK3CA genes in the cohort. (A, B) Lollipop plot shows frequency of somatic mutations of TP53 and PIK3CA in the cohort, respectively. (C) Comparison of the frequency of somatic mutations of recurrently altered amino acid in PIK3CA between Neo-ALL-IN and TCGA postmenoposal ER+HER+ breast cancer study.*

with neoadjuvant combination of letrozole and lapatinib. *TP53* and *PIK3CA* were two most commonly mutated genes in the cohort that were consistently associated with poor clinical outcomes, which showed a significantly higher incidence compared to previous investigations (16, 17). We also observed several interesting genetic aberrations including *MLL2 somatic mutation*, and *RUNX1T1* or MYC amplification, and suggested their potential values for predicting favorable treatment outcomes.

Although the PI3K signaling pathway has been abundantly explored in association with acquired resistance to anti-HER2 or hormonal treatment in BC (18, 19), prognostic or predictive significance of *PIK3CA* mutations is yet undetermined in early ER+HER2+ BC. While the *PIK3CA* mutation alone has not been established as an independent prognostic or predictive biomarker in ER+ BC (20-22), NeoALTTO (23) and NeoSphere (24) consistently endorsed less benefit of anti-HER2 treatment for HER2+ BC carrying *PIK3CA* mutation, particularly in the case of exon 9 mutation in helical domain. Accordingly, in our study, 2 patients who experienced rapid progression or death after neoadjuvant treatment both harbored *PIK3CA* mutations at exon 9 in helical domains, while the majority of *PIK3CA* hotspot mutations in the cohort were observed at exon 20 in kinase domains. Hence, we might carefully speculate that negative predictive impact of *PIK3CA* mutation coud be mediated by hotspot mutations at exon 9 in early ER+HER2+ BC.

MLL2 (*KMT2D*) is a histone methyltransferase involved in chromatin remodeling, and known to function as a tumor-suppressor gene in BC that mostly accompanies inactivating mutations. Given that the activity of *MLL2* as an epigenetic

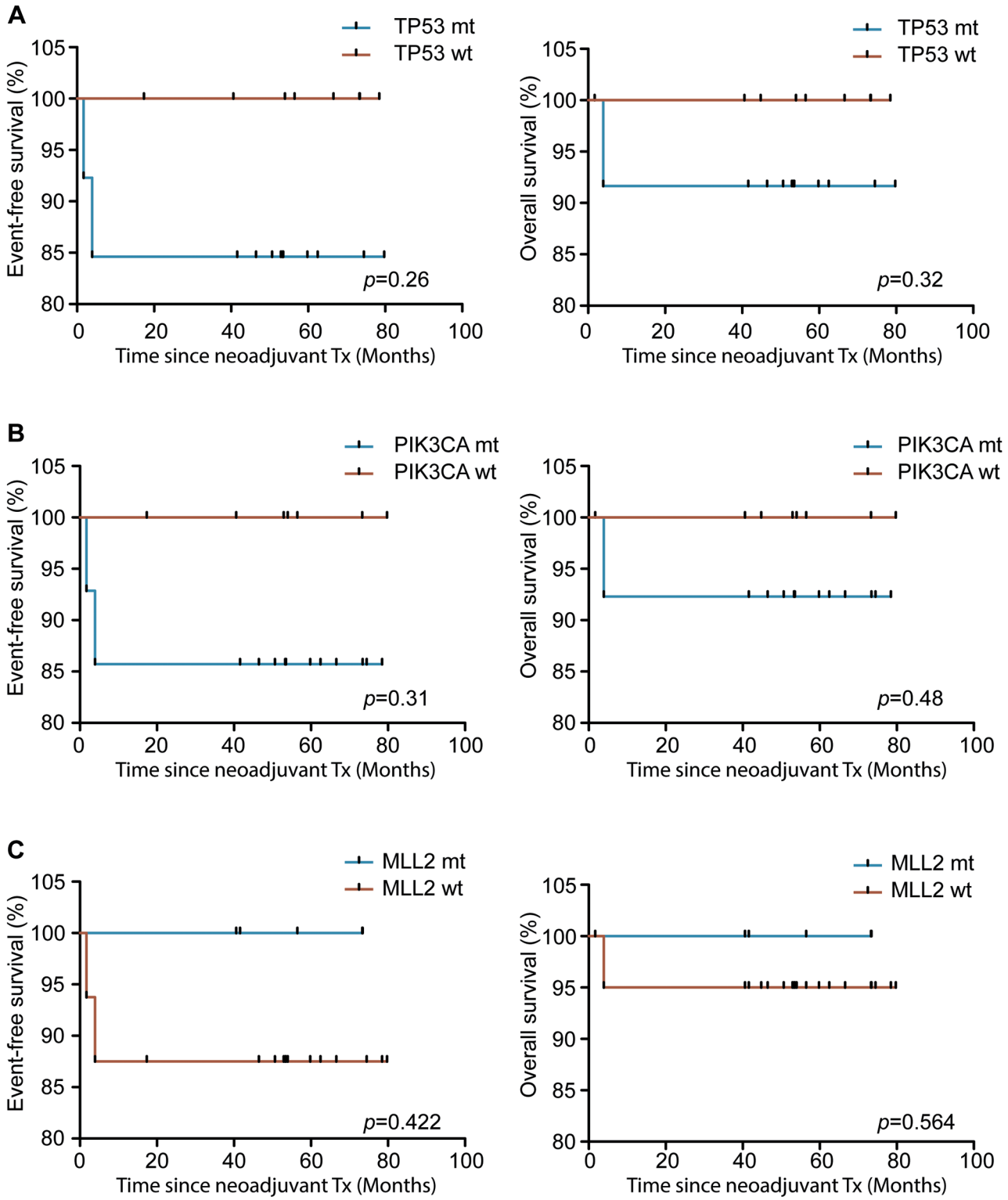

Figure 3. *Comparison of survival outcomes according to frequently identified SNVs. (A) Kaplan-Meir's survival curves presenting event-free survival (EFS) and overall survival (OS) of patients according to the presence of TP53 somatic mutation. (B) Kaplan-Meir's survival curves presenting EFS and OS of patients according to the presence of PIK3CA somatic mutation. (C) Kaplan-Meir's survival curves presenting EFS and OS of patients according to the presence of MLL2 somatic mutation.*

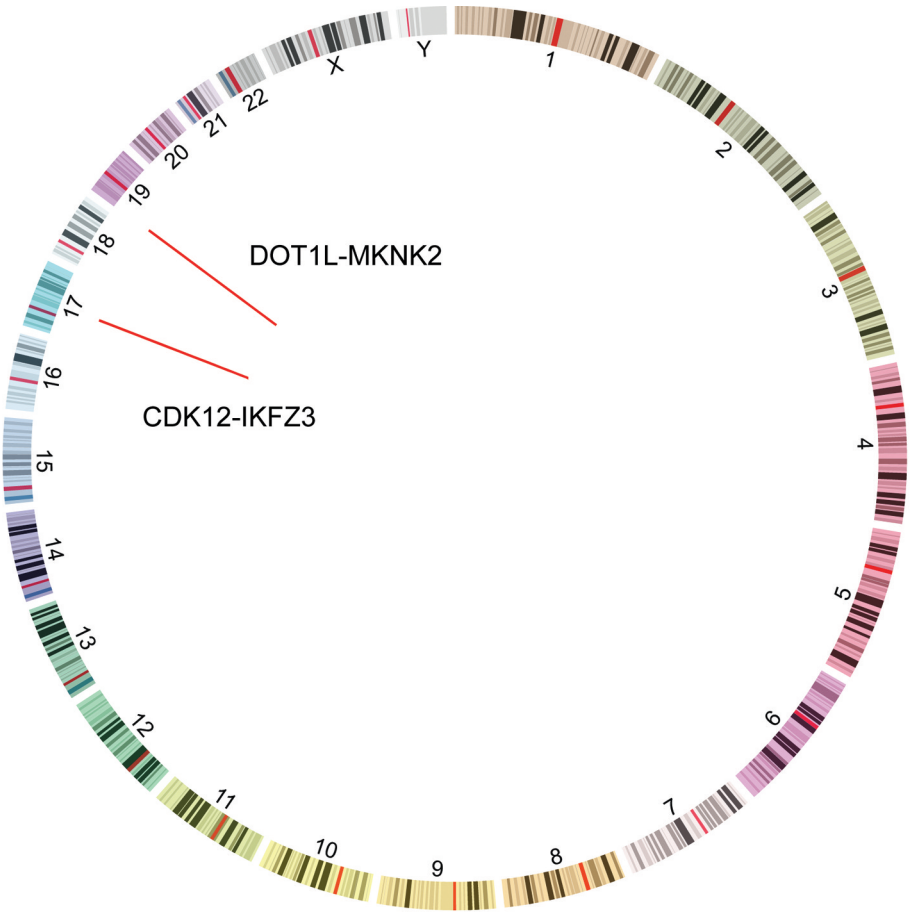

Figure 4. *Structural variations in ER+HER2+ BC.*

player is normally attenuated by AKT in PI3K signaling pathway, its function is enhanced upon PI3Ka inhibition. Once it is activated, it participates in and promotes ER-dependent transcription, which eventually confers resistance to PI3K inhibitors and also endocrine treatment. Accordingly, in recent experimental studies, *MLL2* has been highlighted as an epigenetic regulator of the resistance to treatment with PI3Ka inhibitor in ER+ BC (25, 26). It seems paradoxical at a glance that PI3Ka inhibitor applied to overcome the endocrine resistance might conversely contribute to stimulation of ER signaling. However, it might also represent a potential role of epigenetic regulation to retain the hegemony of homeostasis. According to a preclinical finding that mutation of *MLL2* could reverse stimulated ER signaling *in vitro*, we speculated a plausible hypothesis that inactivating mutation in *MLL2* might prevent the development of endocrine resistance by prohibiting ER-dependent transcription in BCs with a strong ER-signaling dependency. Considering *MLL2* also mediated the development of drug resistance to lapatinib in HER2+ BC cell lines (27), inactivating mutations in *MLL2* might possibly contribute to restoring the sensitivity toward anti-HER2 treatment. Collectively, these all support our findings that *MLL2* somatic mutations were significantly associated with more favorable treatment outcomes and more indolent subset, while it was negatively associated with *PIK3CA* mutations.

Although *RUNX1T1* has been relatively less investigated in BC, it is also a tumor suppressor and translocation partner gene of *RUNX1*, which works as *RUNX1-RUNX1T1* fusion gene during the development of various human cancers (28, 29). Our finding that amplified *RUNX1T1* was associated with better treatment outcomes complies with its potential role as the tumor-suppressor in BC. Interestingly, *MYC* and *RUNX1T1* amplifications were significantly associated, and *MYC*-amplified subset also showed favorable ORR despite their worse survival outcomes. Because amplification of *MYC* in ER+ BC is well-known to mediate the resistance to endocrine therapy (30), better ORR observed in *MYC*-

amplified patients seems intricate to interpret. Because molecular interaction between these two genes has been rarely explored in solid cancers (31, 32), we could only assume it based on a strong correlation between *MYC* and *RUX1T1* observed in the study that favorable ORR could be mainly induced by *RUNX1T1* amplification but not by of MYC gene itself.

Our study has the following limitations. First, as our sample size was small, it inevitably has methodological limitations including selection bias. Thus, molecular signatures with intrinsic prognostic impact, such as *PIK3CA* or *TP53*, could be misregarded as predictive biomarkers. Second, combination of lapatinib and letrozole does not currently place as a standard neoadjuvant treatment for HER2+ BC. Third, biologic difference of Asian women from Western population should be always kept in mind. Substantially a higher incidence of specific mutations including *TP53* and *PIK3CA* compared to preexisting data source (33), could partly be comprehended with the biologic difference between races (34). However, regardless of these limitations, our study still has a value to address potential molecular biomarkers exclusively in ER+HER+ BC.

Taken together, *PIK3CA* hotspot mutations at exon 9 of helical domain, as well as TP53 mutation, might be potential negative predictors in early ER+HER2+ BC treated with neoadjuvant lapatinib and letrozole. In addition, *MLL2* inactivating mutation might confer therapeutic benefit to these patients, potentially by catalyzing an escape from endocrine resistance and enhancing sensitivity to anti-HER2 treatment. These findings all warrant further larger investigation with comprehensive gemonic analysis.

Conflicts of Interest

The Authors have no financial disclosures related to this study.

Authors' Contributions

JHP performed initial data collection and executed the analysis of data, and then drafted the manuscript. DHK made substantial contributions to the methodological review and instruction of genomic analysis including the interpretation of next-generation sequencing results. JK, JHA, and KHJ contributed equally to enrollment of patients as expert medical oncologists of breast cancer, which was fundamentally based on multidiciplinary cooperation with our surgeons BS and SHA, pathologists HJL and GG, radiologist HK and HJS, and nuclear medicine physician DM. Finally, SBK initially inspired the conception and design of the study, and gave final approval of the version to be published. All Authors read and approved the final manuscript.

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