original report

Actionable Gene Alterations in an Asian Population With Triple-Negative Breast Cancer

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abstract

Purpose **It has been suggested that the biologic characteristics of breast cancer may differ among different geographic or ethnic populations. Indeed, triple-negative breast cancer (TNBC), the most lethal breast cancer subgroup, has been reported to occur at a higher incidence in Japan than in the United States. However, most genomic studies of these tumors are from Western countries, and the genomic landscape of TNBC in an Asian population has not been thoroughly investigated. Here, we sought to elucidate the geographic and ethnic diversity of breast cancer by examining actionable driver alterations in TNBC tumors from Japanese patients and comparing them with The Cancer Genome Atlas (TCGA) database, which gathers data primarily from non-Asian patients.**

Materials and Methods **We performed comprehensive genomic profiling, including an analysis of 435 known cancer genes, among Japanese patients with TNBC (n = 53) and compared the results with independent data obtained from TCGA (n = 123).**

Results **Driver alterations were identified in 51 (96%) of 53 Japanese patients. Although the overall alteration spectrum among Japanese patients was similar to that of TCGA, we found significant differences in the frequencies of alterations in *MYC* and *PTK2*. We identified three patients (5.7%) with a high tumor mutational burden, although no microsatellite instability was observed in any of the Japanese patients. Importantly, pathway analysis revealed that 66.0% (35 of 53) of Japanese patients, as well as 66.7% (82 of 123) of TCGA cohort, had alterations in at least one actionable gene targetable by US Food and Drug Administration–approved drug.**

Conclusion **Our study identified actionable driver alterations in Japanese patients with TNBC, revealing new opportunities for targeted therapies in Asian patients.**

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INTRODUCTION

Although breast cancer is one of the most frequently diagnosed cancers among women in the United States and Japan,[1,2] its biologic characteristics may differ among different geographic or ethnic populations. Indeed, breast cancer incidence, peak age of highest incidence, and mortality are different between women in the United States and Japan.[3] Importantly, triple-negative breast cancer (TNBC), the most lethal subgroup of breast cancer and characterized as estrogen receptor negative and progesterone receptor negative with no overexpression of human epidermal growth factor receptor 2/neu,[4] is known to be more prevalent in Japan than in the United States.[5,6]

Large-scale genomic studies based on next-generation sequencing (NGS), such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium, have revealed important driver gene alterations in many types of solid tumors,[7-14] which have led to new targeted therapeutic strategies for precision cancer medicine.[15-19] These studies have also highlighted the geographic and ethnic diversity in the cancer genome.[20,21] However, most NGS-based studies have been conducted in Western countries, where the Asian population is a minority, and the resulting genomic information may not truly represent this population.[8] For instance, only 5.5% of participants have identified themselves as Asians in the TCGA cohort. Therefore, it is of interest to clarify whether there is a genetic difference in TNBC between Asian and Western patients. In our study, we hypothesized that any differences

in genetic driver events may reflect differences in the biologic etiology of TNBC between Asian and Western patients.

MATERIALS AND METHODS

Patients

The Japanese cohort comprised a total of 53 patients diagnosed with stage I to IV TNBC according to the American Joint Committee on Cancer (seventh edition)[22] between 2009 and 2016 at Niigata University Medical and Dental Hospital, Niigata Cancer Center Hospital, Keio University Hospital, Gifu University Hospital, Showa University Hospital, or St Luke's International Hospital. Collection and use of all specimens in this study were approved by the institutional review board of each institution. Informed consent was obtained from all participants. TNBC tumors in both the Japanese cohort and the TCGA database were defined according to previous reports.[23,24]

Sequencing Library Preparation

Archival tissue in the formalin-fixed, paraffin-embedded (FFPE) tumor obtained during routine biopsy or resection of primary tumor was used for analysis. An independent pathologist evaluated the tumor content on hematoxylin and eosin–stained slides for each study sample to ensure > 50% tumor content was present. All sample preparation, comprehensive genomic sequencing, and analytics were performed in a Clinical Laboratory Improvement Amendments/College of American Pathologists–accredited laboratory (KEW, Cambridge, MA) as described previously.[19]

Comprehensive Genomic Sequencing

FFPE genomic DNA (50 to 150 ng) was used to construct libraries that were enriched for 435 genes using CANCERPLEX (KEW) as described previously.[19] The term gene alterations is used when there are genetic aberrations such as amplifications in addition to mutations. To assess the somatic status of alterations in tumor-only settings, we used a filtering strategy similar to one recently published[25] with minor differences. Detailed methodologies including clustering are described in the Data Supplement.

Genomic and Clinicopathologic Data for the TCGA TNBC Cohort

Genomic and clinicopathologic data from a total of 123 tumor samples of TNBC in the TCGA database were downloaded from cBioPortal (www.cbioportal.org). *BRCA* alteration data for the TCGA TNBC samples were downloaded from the Broad GDAC Firehose Web site (https://gdac.broadinstitute.org/). As in the 435-gene panel bioinformatics pipeline, silent mutations that did not alter the amino acid sequence were removed from the data set. To compare the mutational burden of the 435-gene panel with that of the TCGA whole-exome sequencing (WES) data, the data set of single-nucleotide polymorphisms was downsampled to the 435 genes in the panel, and the panel mutation rate was determined, calculated as mutations per megabase, as described previously.[19]

RESULTS

Genetic Alterations in Japanese Patients With TNBC

The clinicopathologic characteristics of the patients with TNBC in the Japanese and TCGA cohorts are summarized in the Data Supplement. The Japanese cohort included more patients with M1 disease (11%) compared with the TCGA cohort (1%; $P < .01$), which seemed to affect the difference in stage among the two cohorts (Data Supplement). After performing comprehensive genomic sequencing on all Japanese TNBC samples, we found at least one alteration in 82 of 435 cancer-associated genes (Fig 1; Data Supplement). Although a total of 186 alterations were found in the 53 patients, there were only three frequently altered genes (seen in > 10% of patients): *TP53*, *PIK3CA*, and *PTEN* (Fig 1). Alterations of *BRCA1* and *BRCA2* presented in 9.4% and 5.6% of patients, respectively, totaling 15.1% of the population (Fig 1).

The Japanese cohort included 35 patients who received neoadjuvant chemotherapy. Among them, biopsy specimens of primary tumors before neoadjuvant chemotherapy were used for analysis in 11 patients, and surgical specimens of remnant primary tumors after neoadjuvant chemotherapy were used in 24 patients (Data Supplement). We compared gene alterations in chemotherapy-naïve tissue samples (n = 29) and chemotherapy-affected tissue samples (n = 24) and found that the gene alteration frequencies,

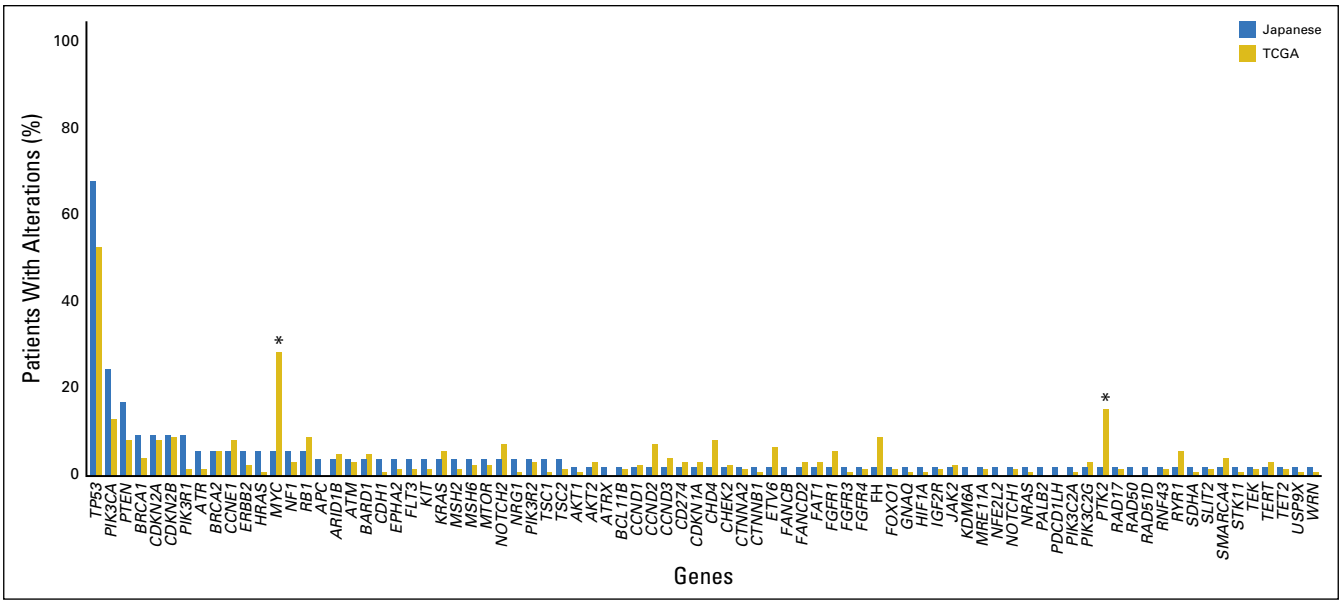

Fig 1. Comparison of genetic alterations in triple-negative breast cancer between the Japanese and The Cancer Genoma Atlas (TCGA) cohorts. Alteration frequencies of cancer-associated genes in Japanese patients detected by a 435-gene panel, and alteration frequencies for the same gene set in the TCGA cohort. Statistical significance was determined using Fisher's exact test. (*) $P < .05$ for alteration frequencies between Japanese patients and the TCGA cohort.

including those for *TP53*, *PIK3CA*, and *PTEN*, were comparable between the two groups (Data Supplement). We also extracted gene alteration data from 123 cases in the TCGA TNBC cohort for the same set of genes as in Japanese patients to compare our results with TCGA data (Fig 1; Data Supplement). Of note, there were two distinct categories in the TCGA cohort: TNBC based on pathologic subtyping and basal-like breast cancer based on PAM50 analysis, with some overlap between the two groups. Interestingly, the *TP53* alteration rate in TNBC (65 [52.8%] of 123) was relatively low in the TCGA cohort, although the rate in basal-like breast cancer (73 [78.5%] of 93) was much higher in the same cohort (Data Supplement). Generally, the gene alteration rates were comparable between Japanese patients and the TCGA cohort; however, the alteration frequencies in two genes (*MYC* and *PTK2*) were significantly different between the two cohorts (Fig 1; Data Supplement).

Genomic Alterations in Cancer Signaling Pathways

We next assessed genomic alterations in major oncogenic pathways involving phosphatidylinositol 3-kinase, the cell cycle, DNA double-strand break (DSB) repair, ERBB2/KRAS, and β-catenin/WNT signaling and determined whether there were differences between the two cohorts (Figs 2A to 2J). Overall, the gene alteration spectrum of the Japanese cohort was similar to that of the TCGA cohort (Figs 2A to 2J). However, there were some exceptions, such as significantly more amplification of *MYC* in the TCGA cohort (28%) than in Japanese patients (6%; $P < .01$; Fig 2).

We found that eight patients (15%) showed *BRCA1/2* alterations, including five *BRCA1* and three *BRCA2* alterations (Figs 2A to 2J). There was no difference in the frequency of *BRCA1/2* alterations between the Japanese and TCGA cohorts (Figs 2K to 2O). We further analyzed the *BRCA1/2* alterations in the Japanese cohort (Table 1). On the basis of the alteration site, seven of eight alterations were considered to be pathogenic alterations (Table 1). Moreover, bioinformatic analysis revealed that seven of eight alterations were possible germ line alterations, based on the allelic fraction and alteration site compared with a database, such as ClinVar (Table 1). We did not confirm the germ line alteration using patients' normal tissue.

Tumor Mutational Burden and Microsatellite Instability in Patients With TNBC

A high tumor mutational burden (TMB-H) tumor is defined as a tumor with a high rate of somatic mutation. TMB-H tumors were recently correlated with the generation of neoantigens and clinical response to immunotherapy drugs.[7,26-29] The median mutation rate for Japanese patients was 11.5 mutations/Mb (range, 3.9 to 56.2 mutations/Mb). We identified three

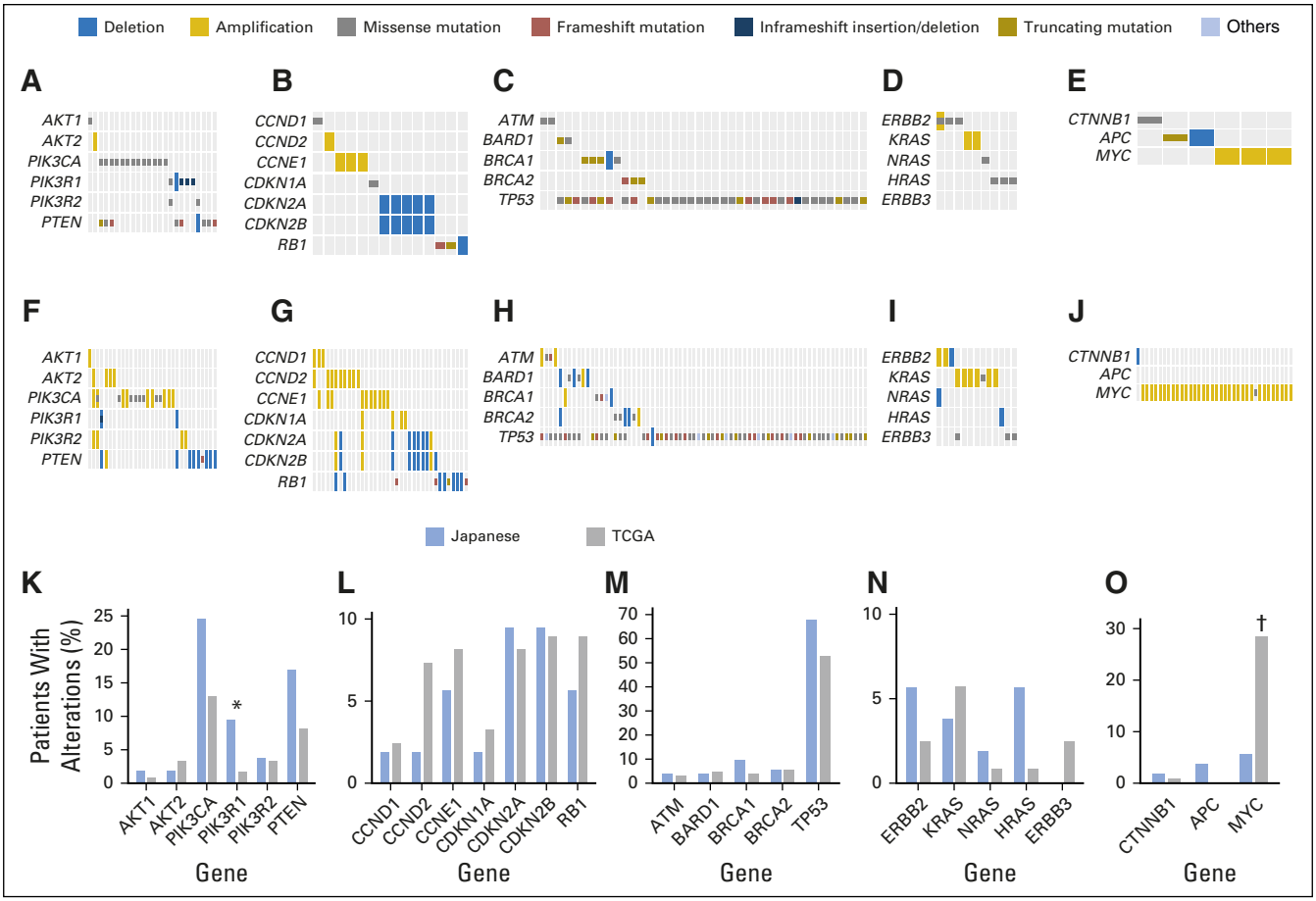

Fig 2. Genetic alterations across common oncogenic pathways in triple-negative breast cancer. (A-E) Japanese patients (n = 53) and (F-J) The Cancer Genome Atlas (TCGA) cohort (n = 123) were evaluated for gene alterations in key cancer pathways: (A, F) phosphatidylinositol 3-kinase (PI3K; 45% and 25% of patients, respectively), (B, G) cell cycle (26% and 29% of patients, respectively), (C, H) DNA double-strand break (DSB) repair (75% and 58% of patients, respectively), ERBB2/KRAS (17% and 11% of patients, respectively), and (E, J) β-catenin/WNT (11% and 29% of patients, respectively). Altered cases are defined as the total number of unique samples with a genetic alteration in each pathway. (K-O) Percentage of patients with a variation for each given gene: (K) PI3K, (L) cell cycle, (M) DNA DSB repair, (N) ERBB2/KRAS, and (O) β-catenin/WNT. Statistical significance was determined using Fisher's exact test. (*) $P < .05$. (†) $P < .01$.

patients (5.7%) as TMB-H (≥ 20 mutations/Mb of sequenced DNA). Of note, one of the three TMB-H patients showed *BRCA1* alteration (Table 1). This classification was based on our previous studies of colorectal and gastric cancers, which included a number of patients with microsatellite instability, although no microsatellite instability was observed in any of the 53 Japanese patients with TNBC (Fig 3A). To compare the mutational burden in Japanese patients with that in the TCGA cohort, we downsampled the TCGA WES data (n = 123 tumors) to the subset of 435 genes in the panel test platform. This analysis not only accurately identified TMB-H tumors but also showed strong correlation in mutation rates between the 435-gene panel and WES (R^2 = 0.9203; Fig 3B). The average mutation rate detected by the 435-gene panel (8.6 mutations/Mb) was higher than that detected by WES (1.7 mutations/Mb), reflecting the fact that the panel content was in part selected to include genes more frequently mutated in cancer, as reported previously.[19] The mutational burden of the TCGA TNBC cohort reanalyzed in silico revealed a comparable mutational burden between the Japanese cohort and the TCGA cohort, in which six (4.9%) were determined as TMB-H (Fig 3).

Clustering Analysis for Gene Alterations in Patients With TNBC

Next, we performed hierarchic clustering of alterations in a subset of genes associated with an US Food and Drug Administration (FDA) –approved therapy (on or off label; n = 68 genes) across all 176 TNBC samples (Japanese,

Table 1. *BRCA1* and *BRCA2* Alterations in Japanese Patients With TNBC (n = 53)

Sample	Gene	Alteration	Type	Allelic Fraction	Effect	Possible Germ Line	Hypermutation
D0576	*BRCA1*	Q934X	Truncating	0.31	Pathogenic*	Yes*	No
D0577	*BRCA2*	Q969X	Truncating	0.34	Pathogenic†	Yes†	No
D0586	*BRCA2*	W3127fs	Truncating	0.46	Pathogenic†	Yes†	No
D0594	*BRCA1*	D1337fs	Truncating	0.79	Pathogenic†	Yes†	No
D0604	*BRCA1*	Q934X	Truncating	0.65	Pathogenic*	Yes*	No
D0718	*BRCA2*	R2520X	Truncating	0.87	Pathogenic‡	Yes‡	No
D0733	*BRCA1*	p.F1662S	Missense	0.48	VUS/likely benign/ benign§	Yes§	Yes
D0736	*BRCA1*	Genomic loss	Loss	Likely heterozygous deletion	Pathogenic	NA	No

Abbreviations: NA, not applicable; TNBC, triple-negative breast cancer; VUS, variant of unknown significance.
*The alteration has been seen in germ line (ClinVar identifier RCV000077528.3) and somatic (cBioPortal).
†The alteration has not been described before.
‡The alteration has been seen in germline (ClinVar identifier RCV000162645.1) and somatic (cBioPortal).
§Classified as VUS or benign in ClinVar (identifiers RCV000048718.2, RCV000112441.2, RCV000130003.2). This missense variant lies in the first BRCT domain. It has been reported in association with hereditary breast cancer and has been classified as VUS or benign variant upon review by expert panel based on in silico study (ClinVar; PubMed identifier 21990134).

n = 53 plus TCGA, n = 123) to further assess the characteristics of genomic alterations in TNBC (Fig 4). We determined that all patients could be classified into 10 typical clusters (Fig 4B). Cluster one (n = 83; 47.2%), in which no common actionable alterations were seen, was the largest of the 10 identified clusters, and cluster nine (n = 23; 13.1%), in which *PIK3CA* alterations were common, was the second largest cluster (Fig 4B). Cluster seven (n = 16) was characterized by *PTEN* alterations, cluster eight (n = 12) was characterized by *CDKN2A* and *CDKN2B* alterations, and clusters six (n = 6) and three (n = 6) were characterized by *BRCA1* and *BRCA2* alterations, respectively. Moreover, cluster two (n = 7) contained *FGFR1* alterations, cluster four (n = 7) contained *DDR2* alterations, and cluster five (n = 10) contained *CCND2/3* alterations. Finally, cluster 10 (n = 6) was characterized by multiple gene alterations, including *PIK3CA*, *PTEN*, and *CDKN2A/B*. In summary, the large cluster one, without any distinct alterations, and other clusters with typical alternated genes highlighted the great diversity of alterations underlying TNBC, which consequently constrained our clustering analysis designed to match genomic alterations with FDA-approved drugs.

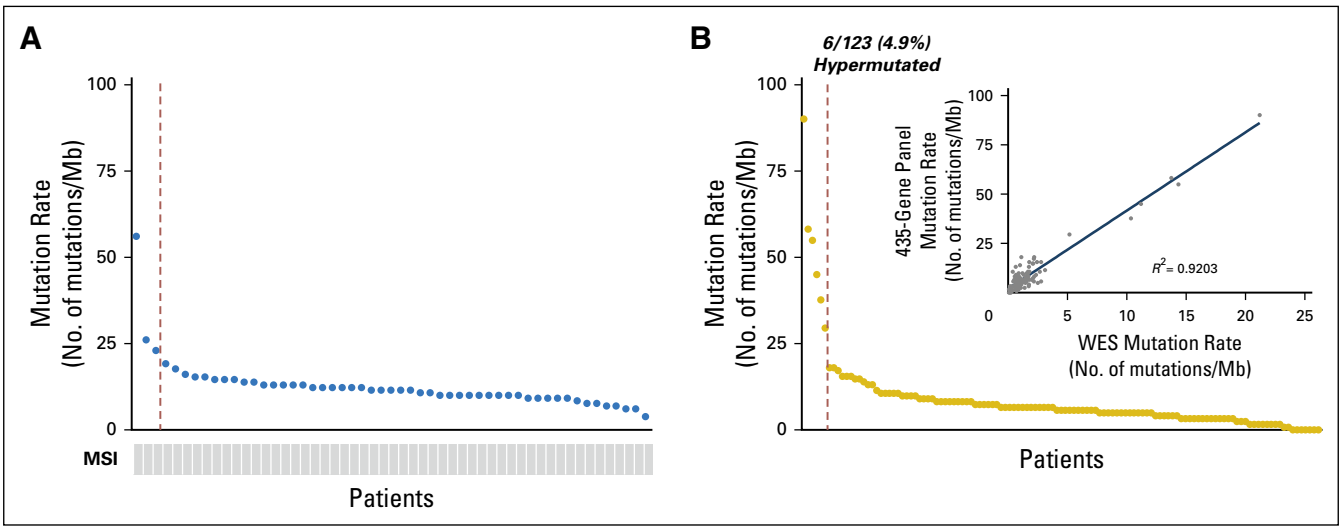

Fig 3. Genetic alterations related to potential targeted therapies. (A) Mutation rate and microsatellite instability (MSI) in patients with triple-negative breast cancer in Japan (n = 53); three (5.7%) of 53 hypermutated. (B) Mutation rate data from whole-exome sequencing (WES) for The Cancer Genome Atlas cohort (n = 123) were downsampled to the content of the 435-gene panel platform; six (4.9%) of 123 hypermutated. Inset: correlation between mutation rates determined by 435-gene panel and WES.

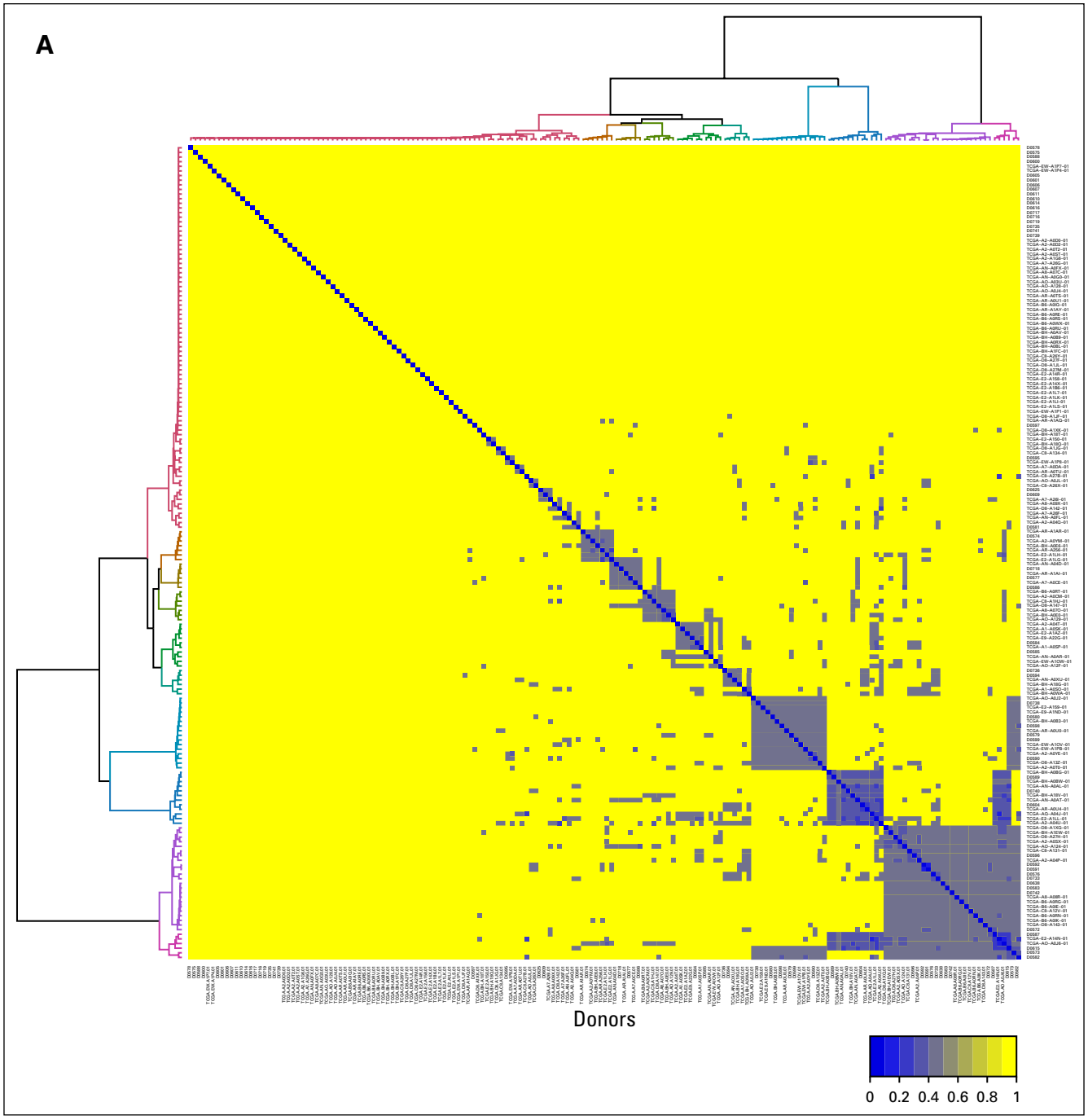

Fig 4. Cluster of 68-gene coalteration patterns. (A) A cluster analysis was performed on 176 cases of triple-negative breast cancer (TNBC; 53 tumors from Japanese patients and 123 tumors from The Cancer Genome Atlas [TCGA] cohort) by using Euclidean distance and Ward's clustering method (distance based on common mutated genes are colored blue to yellow). The 68 genes associated with a US Food and Drug Administration–approved drug (on or off label) were used for clustering.

Actionable Alterations for Potential Treatments

Because the cluster analysis did not effectively identify potential treatment strategies on the basis of individual genomic alterations, we therefore assessed the possibility of using pathways associated with FDA-approved drugs to identify targeted therapies for patients with TNBC. We determined that 29 of 82 mutated genes were actionable with FDA-approved drugs (on or off label). Importantly, 35 patients (66.0%) had at least one potentially actionable alteration associated with FDA-approved drugs, whereas 18 patients had no actionable alterations with those

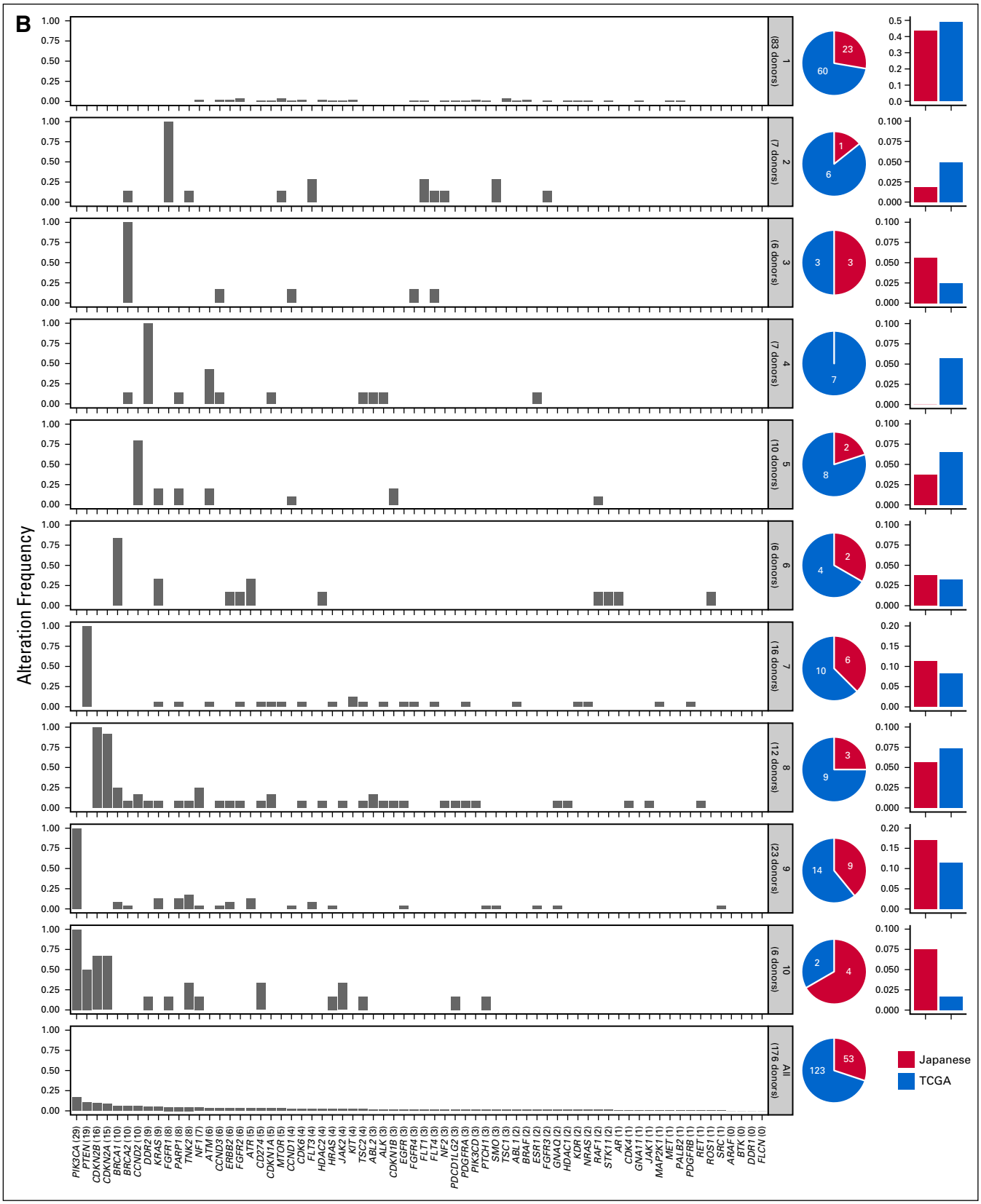

Fig 4. (Continued). (B) Comutated gene patterns of a 68-gene set. The percentage of Japanese patients and the percentage of the TCGA cohort in each cluster are shown in the pie graphs displayed on the right side. The alteration rate in each cluster is shown as a bar graph in the far right.

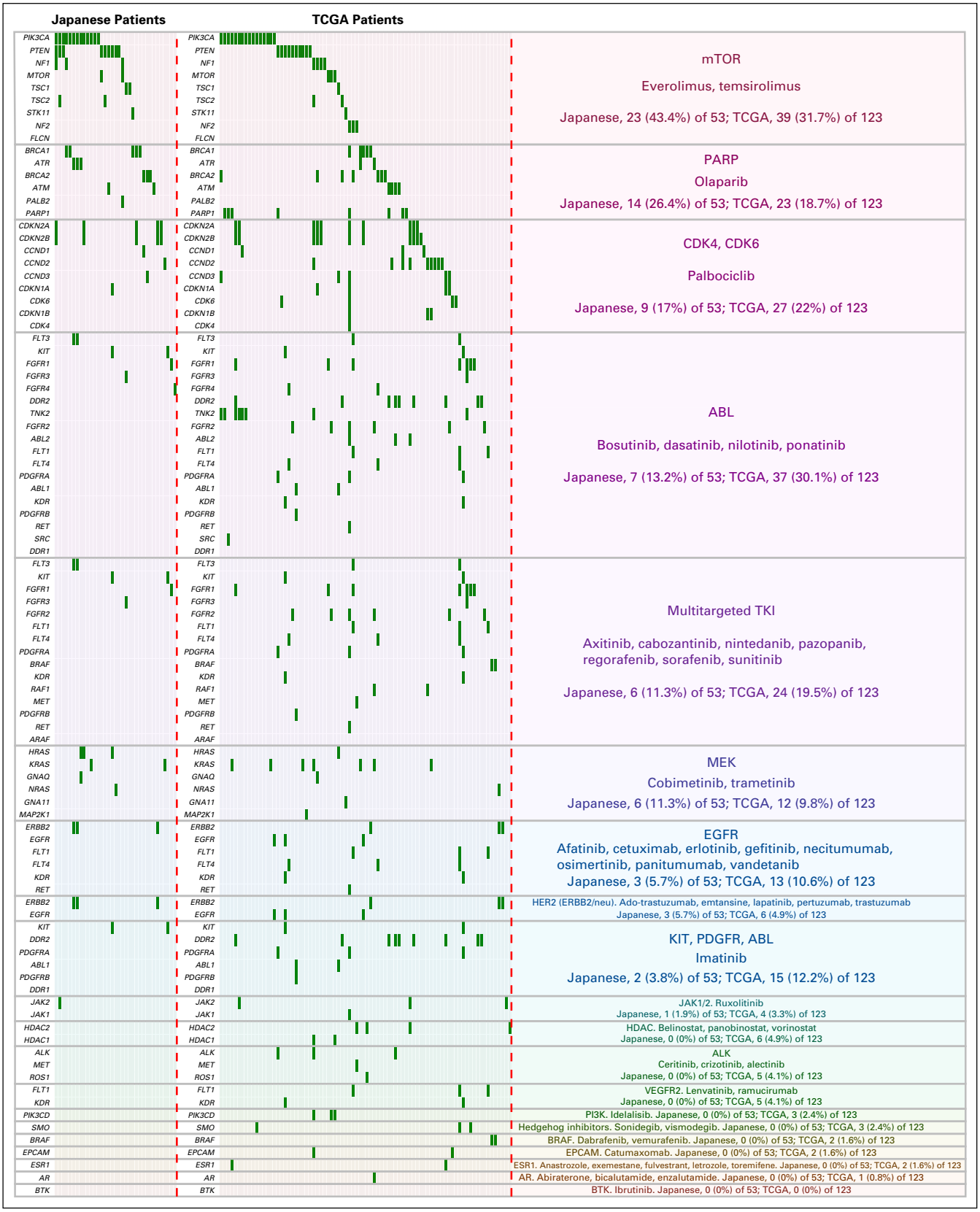

Fig 5. Genetic alterations in Japanese patients with triple-negative breast cancer and patients in The Cancer Genome Atlas (TCGA) cohort potentially targetable by US Food and Drug Administration (FDA) –approved cancer therapies. The genes associated with an FDA-approved drug (on or off label) are listed, and each individual genetic alteration is indicated as a green bar. The FDA-approved cancer therapies are shown in the right column. The alteration rates in the Japanese population (n = 53; alterations, 35 [66%] of 53) and TCGA cohort (n = 123; alterations,

drugs (Fig 5). Interestingly, we similarly analyzed the TCGA cohort and found that, as with the Japanese cohort, 66.7% of cases had at least one potentially actionable alteration associated with FDA-approved drugs.

For Japanese patients, genes associated with mammalian target of rapamycin (mTOR), poly (ADP-ribose) polymerase (PARP), and CDK4/6 inhibitors showed the three highest alteration rates (Fig 5). Twenty-three patients (43.4%) showed actionable alterations that could be targeted by mTOR inhibitors, whereas 31.7% of TCGA cases showed actionable alterations in this pathway. Importantly, 26.4% of Japanese patients and 18.7% of TCGA cases had actionable alterations related to the DSB repair pathway, which could be potentially treated by PARP inhibition. Seventeen percent of Japanese patients and 22% of TCGA cases had alterations in actionable genes related to the cell-cycle pathway, which could be treated by CDK4/6 inhibition. Moreover, other FDA-approved drugs, including multitargeted tyrosine kinase inhibitors, MEK inhibitors, anti–human epidermal growth factor receptor 2 therapies, and JAK inhibitors, were indicated as potential targeted therapies for Japanese patients with TNBC, with even more drugs suggested for TCGA cases (Fig 5). Of note, Oncoprint analysis showed coalterations in many cases of TNBC in both cohorts, which may be associated with resistance to the targeted therapies (Fig 5). Additional investigation, including clinical outcomes, is needed to reveal the efficacy of these therapies for patients with TNBC.

DISCUSSION

We found significant differences in the frequencies of gene alterations, including *MYC* amplification, between Japanese patients with TNBC and the TCGA TNBC cohort. However, the overall alteration spectrum of the Japanese patients was similar to that of the TCGA cohort.[8] Importantly, 66% of Japanese patients, as well as 67% of TCGA cases, had at least one genomic alteration in actionable genes, providing potentially new treatment strategies.

We demonstrated significant differences in the frequencies of alterations in two genes: *PTK2* and *MYC*. It has been previously reported that *MYC* is one of the most important driver genes, and there are known ethnic differences in *MYC* amplification frequency.[30] Although we found a *MYC* amplification rate of 5.7% in our cohort, high incidence (30% to 35%) of *MYC* amplification has been repeatedly reported from US groups, similar to TCGA rates.[31-33] A unique patient population comprises the cohort described in our study, which could be useful given the sparse genomic data from Asian TNBC populations.

We analyzed genomic alterations in FFPE tumor tissue from a cohort of Japanese patients with TNBC by comprehensive NGS-based sequencing of a panel of 435 cancer-relevant genes, at an average 500× depth. In contrast, TCGA data were obtained by WES of fresh frozen tumor tissue, with a mean coverage of 97.6× depth. FFPE samples are more commonly available than fresh frozen samples. Method of preservation is critical for DNA analysis using FFPE tissue samples, as we reported previously.[34] The quality of all DNA samples used in this study was confirmed to be adequate for accurate NGS analysis by strict quality control checks. Indeed, most of the genes altered in both the Japanese and TCGA cohorts showed similar frequencies, consistent with previous reports, despite the different sequencing techniques used.

Previous comprehensive genomic profiling studies have reported that TNBC is a heterogeneous disease involving diverse genomic alterations.[8,35,36] Our results also demonstrated the heterogeneity of TNBC, with only three genes mutated in > 10% of patients and most of the genetic alterations differing from one another. Moreover, clustering analysis of patients with TNBC on the basis of genetic alterations also emphasized the heterogeneous nature of TNBC. Because of this heterogeneity, it was difficult to find common actionable driver alterations for therapies, and our results demonstrated that a

Fig 5. (Continued)
82 [66.7%] of 123) are also shown in the right column. AR, androgen receptor; BTK, Bruton tyrosine kinase; EGFR, epidermal growth factor receptor; EPCAM, epithelial cell adhesion molecule; HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositol 3-kinase; TKI, tyrosine kinase inhibitor; VEGFR2, vascular endothelial growth factor receptor 2.

more comprehensive approach, such as the one used in this study, will be required to find actionable alterations in each patient with TNBC.

To explore the clinical utility of targeted sequencing with a large panel for TNBC, we identified actionable genes that can be treated by FDA-approved drugs and found that 35 Japanese patients (66%) had at least one such actionable gene alteration. We identified several signaling pathways for molecular targeted therapies, including the PI3K pathway for mTOR inhibitors, the DSB repair pathway for PARP inhibitors, and the cell-cycle pathway for CDK4/6 inhibitors (Fig 5). These important pathways in Japanese TNBC are comparable to those in previous reports from Western countries,[8,31,36,37] and potential targeted therapies for these pathways could be similar, regardless of ethnicity.[18,38]

In addition to identifying driver alterations, the clinical significance of identifying hypermutated tumors was recently demonstrated in several studies correlating mutational burden with the development of neoantigens and clinical response to immunotherapy drugs.[7,26-29,39] We previously showed that a panel of 400+ genes was able to identify TMB-H tumors with sensitivity and specificity comparable to those of WES in colorectal cancer.[19] Similarly, here we determined the TMB and revealed that a small proportion of patients (5.7%) were hypermutated. Although none of these patients received immune checkpoint inhibitor therapy, there is a high likelihood that these patients may have responded, given clinical data demonstrating efficacy in TMB-H patients.

In our study, we found that seven (13%) of 53 Japanese patients with TNBC had possible germ line *BRCA1/2* alterations based on bioinformatic analysis of tumor DNA. Although specific genetic tests are needed to confirm the frequency of germ line *BRCA1/2* alterations, this estimated frequency is in agreement with previous reports.[40] Recently, germ line alteration of *BRCA1/2* has attracted increasing attention because of the use of PARP inhibitors. Considering the frequency of germ line *BRCA1/2* alterations, it seems that there is a certain population that would benefit from treatment with PARP inhibitors, not only in Western countries, but also in Asian countries, including Japan.

Patients were approached consecutively. However, the number of patients enrolled in this study was limited, partly because of certain misunderstandings and prejudices that prevent some Japanese patients consenting to genomic sequencing. Power analyses for all genes were not conducted before this study; however, we did verify the test power based on gene alteration rates of 53 Japanese samples. As a result, we determined that a Fisher's exact test of significant genes *MYC* and *PTK2* met adequate power (≥ .8). Therefore, we considered 53 as a sufficient number for the Japanese cohort, at least to the extent that the alteration rate of more than one gene was significantly different between the Japanese and TCGA cohorts.

Another limitation is that we included patients receiving neoadjuvant chemotherapy, and we cannot exclude the possibility that the neoadjuvant chemotherapy affected genomic alterations. However, when we compared the gene alterations between the chemotherapy-naïve tissue samples (n = 29) and chemotherapy-affected tissue samples (n = 24), we found that gene alteration frequencies, including those for *TP53*, *PIK3CA*, and *PTEN*, were comparable between the two groups (Data Supplement). Moreover, considering that we did not see major differences in genomic alterations between the Japanese and TCGA cohorts except for *MYC*, it is unlikely that neoadjuvant chemotherapy dramatically influenced the landscape of driver alterations. Although it is not easy yet in Japan to recruit a large number of patients with TNBC for genomic sequencing, additional studies with a greater number of patients are warranted to confirm our findings in the future.

Despite these limitations, to our knowledge, this is the largest cohort of Japanese patients with TNBC to undergo comprehensive genomic profiling to date, and our data provide important insights into the molecular heterogeneity underlying this aggressive disease from an ethnogeographic perspective. Similarly, several studies have demonstrated the heterogeneity of TNBC,[5,41-43] therefore suggesting that targeting TNBC by molecular targeted therapies remains a challenge. Indeed, our results showed coalterations in many TNBC cases in both cohorts, potentially associated with resistance to targeted therapies. Although our study indicates the possibility of targeted therapies for patients with TNBC, an accumulation of clinical evidence will be required to reveal the usefulness of each therapy for this disease. Additional clinical studies

are required to reveal potential treatments for patients with TNBC.

In conclusion, our study revealed actionable driver alterations in Japanese patients with TNBC that could potentially be treated with existing drugs. Although we found significant differences in the frequencies of gene alterations between Japanese and TCGA TNBC patients, which included *MYC* amplification, potential targeted therapies for patients with TNBC could be similar regardless of ethnicity. Our study has revealed new opportunities for the use of targeted therapies in the Asian population.

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Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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