TERT promoter hotspot mutations in breast cancer

Tatsunori Shimoi[1,2], Masayuki Yoshida[3], Yuka Kitamura[1], Tomomi Yoshino[1], Asuka Kawachi[1], Akihiko Shimomura[1], Emi Noguchi[1], Mayu Yunokawa[1], Kan Yonemori[1], Chikako Shimizu[1], Takayuki Kinoshita[4], Koichi Ichimura[5], Takahiro Fukuda[2,6], Yasuhiro Fujiwara[1], Kenji Tamura[1*]

[1] Department of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo, Japan

[2] Course of Advanced Clinical Research of Cancer, Juntendo University, Graduate School of Medicine, Tokyo, Japan

[3] Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan

[4] Department of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

[5] Division of Brain Tumor Translational Research, National Cancer Center, Tokyo, Japan

[6] Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

*Corresponding author:

Kenji Tamura, MD. PhD.,

Department of Breast and Medical Oncology,

National Cancer Center Hospital,

5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan.

Tel: 81-3-3542-2511;

Fax: 81-3-3542-3815;

Email: ketamura@ncc.go.jp

Abstract

Background Telomerase reverse transcriptase (*TERT*) promoter mutations have been discovered in solid and hematological malignancies, where they reflect *TERT* activation and cell-cycle progression. In melanoma, glioma, and thyroid cancers, *TERT* promoter mutations are associated with a poor prognosis. However, no studies have evaluated the prevalence and prognostic significance of *TERT* promoter mutations in breast cancer.

Methods We analyzed *TERT* promoter hotspot mutations (C228T and C250T) using direct sequencing of DNA from 319 tumor tissues. We also collected clinical data from cases that were positive for *TERT* promoter mutations.

Results We detected *TERT* promoter mutations in three (0.9%) of the 319 cases. Two patients had hormone receptor-positive and human epidermal growth factor receptor 2-negative cancer, while the third patient had triple-negative cancer. All three patients had initially been diagnosed with operable breast cancer and undergone surgical treatment. The relapse-free survivals of these patients were 83, 226, and 270 months, respectively. The mutations were C250T in the triple-negative cancer case and C228T in the remaining two cases.

Conclusion Given the rarity of *TERT* promoter mutations, further studies are needed to confirm their prognostic significance in breast cancer cases.

Keywords: Breast cancer, telomerase reverse transcriptase, *TERT* promoter mutation,

Introduction

Breast cancer is a common cancer and the leading global cause of cancer-related mortality among women [1]. In addition, breast cancer is a heterogeneous condition that is categorized into four subtypes according to pathological review, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) status. Furthermore, using whole-genome sequencing, numerous somatic and driver mutations have been identified in breast cancer [2]. This information has allowed physicians to stratify patients according to their tumor's molecular characteristics and select appropriate therapies. Recent clinical trials have indicated that mutations in the genes for phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) and RAC-alpha serine/threonine-protein kinase (*AKT1*) may predict the response to PI3K and AKT inhibitors, respectively [3–5].

Telomerase is a DNA polymerase that maintains the length of telomeres at the end of chromosomes. Its activity is relatively high in stem cells and is downregulated in normal somatic cells. However, genetic mutations can also affect the non-coding regulatory region of the telomerase reverse transcriptase (*TERT*) gene's promoter, and telomerase can be activated by mutations in the *TERT* promoter. Many malignant tumor cells have TERT expression or telomerase activity, with >90% of breast cancer cases

having telomerase activity [6].

In 2013, somatic hotspot mutations in the promoter region of *TERT* were reported among patients with melanoma [7,8]. In addition to these two common hotspots identified on chromosome 5: 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T), the tandem mutations of 1,295,228/1,295,229 CC>TT (C228T/C229T) and 1,295,242/1,295,243 CC>TT (C242T/C243T) have also been reported in melanoma with *TERT* promoter mutation. In a previous report, mutation frequencies for C228T, C250T, C228T/C229T and C242T/C243T in melanoma cell lines were 46/168 (27%), 64/168 (38%), 7/168 (4.2%) and 8/168 (4.8%), respectively. On the contrary, no mutation sites other than C228T and C250T have been reported in glioma. In one large retrospective cohort, the mutation frequencies of C228T and C250T in primary glioblastoma with *TERT* promoter mutation were 123/256 (48%) and 56/256 (22%), respectively [13]. Since the first report of melanoma, >30 types of tumors have been found to contain *TERT* promoter mutations, hepatocellular carcinoma (41%), thyroid cancer (11–43%), ovarian clear cell carcinoma (16%), bladder cancer (63%), and phyllodes tumors of the breast (65%) [9,10,11]. In these types of cancer other than melanoma, C228T and C250T were also hotspots of *TERT* promoter mutations, in which C228T is more dominant than C250T. These hotspot mutations are associated

with a poor prognosis [7,12,14–15]. However, to the best of our knowledge, no reports have evaluated the prevalence and prognostic significance of *TERT* promoter mutations in human breast cancer. Therefore, the present study aimed to provide information regarding *TERT* promoter mutations in samples from human breast cancer.

Materials and Methods

We retrospectively evaluated 319 breast cancer specimens (125 frozen specimens and 194 formalin-fixed paraffin-embedded [FFPE] specimens) that were provided by the National Cancer Center Biobank of Japan and had been obtained between January 1983 and November 2015. Three patients had metachronous breast cancer and we included all of their samples in this study. We extracted DNA from the frozen specimens using the QIAamp DNA FFPE tissue kit (QIAGEN, Tokyo) and from the FFPE specimens using the QIAamp DNA Mini Kit (QIAGEN, Tokyo), according to the manufacturer's recommendations. The *TERT* promoter mutations (C228T and C250T) were identified using direct sequencing according to the previously reported methods [12,13], and the direct sequencing was performed by FASMAC (Tokyo, Japan). The forward primer's sequence was 5'-gtaaaacgacggccagcaggaaacagctatgacccagctccgcctcctccg-3' and the reverse primer's

sequence was 5'- gctgcctgaaactcgcgcc-3'. Mutations in the *PIK3CA* gene (E542K, E545K, or H1047R) were detected using quenching probe system by the i-densy IS-5320 system (ARKRAY Inc., Kyoto, Japan) [16].

Estrogen receptor (ER) and progesterone receptor (PgR) were classified positive if ≥10% of tumor cells demonstrated positive nuclear staining for ER or PgR, respectively [17]. HER2 positivity was classified according to the recommendation of the American Society of Clinical Oncology/College of American Pathologists guidelines [18]. Hormone receptor positive was defined as positive for ER or PgR. Histological and nuclear grades were reported according to previously reported criteria [19, 20].

Relapse-free survival was defined as the time between the day of diagnosis and disease progression or last follow-up. Overall survival was defineed as the time between the day of diagnosis and the day of death or last follow-up.

This study's retrospective protocol was approved by the National Cancer Center Institutional Review Board (No. 2014-092). Written informed consent was not obtained from the patients. The results of this study have been published on our hospital's web page.

Results

Sequencing was successful for all 319 specimens, and we detected *TERT* promoter mutations in three samples (0.9%). The *TERT* promoter mutations were only detected in the tumor tissue DNA and were not detected in the three patients' normal tissue DNA. The three patients' characteristics are shown in Table 1. All patients were women who had experienced a relatively late relapse, after undergoing surgery because of an early diagnosis of breast cancer. Two patients demonstrated relapse-free survivals of >200 months. The histological results were invasive ductal carcinoma in two cases and invasive lobular carcinoma in one case. Two patients had hormone receptor-positive and HER2-negative breast cancer, while one patient had triple-negative breast cancer (TNBC). C250T mutations were noted in the TNBC case and C228T in the two hormone receptor-positive cases (Figure 1). Two of these patients had mutations in the *PIK3CA* kinase domain (H1047R) (Table 1).

Discussion

This is the first detailed report regarding *TERT* promoter mutations in human breast cancer, and we identified *TERT* promoter mutations in three (0.9%) of 319

samples. However, a previous report identified two cases with *TERT* promoter mutations among eight human breast cancer cell lines (25%) [12]. Nevertheless, another report failed to detect *TERT* promoter hotspot mutations in 88 breast cancer samples [10]. In the present study, we found that two of the three samples with *TERT* promoter mutations also had *PIK3CA* kinase domain mutations. In ovarian cancer, *TERT* promoter mutation and *PIK3CA* mutation are considered mutually exclusive [21], whereas this coexistence is reported in other carcinomas [22–23]. Given that *PIK3CA* mutation is a relatively common mutation in breast cancer, we investigated the coexistence of these mutations. Interestingly, *PIK3CA* mutations only exist in approximately 30% of patients with hormone receptor-positive breast cancer and in approximately 10–20% of TNBC cases. Moreover, *TERT* promoter mutations coexist with *PIK3CA* mutations in 12% of anaplastic thyroid cancers [22], but only in 7.5% of gliomas [23]. Thus, it is possible that a relationship exists between *TERT* promoter and *PIK3CA* kinase domain mutations.

TERT promoter mutations occur during the early stage of glioma or thyroid cancers and are associated with a poor prognosis among patients with melanoma, glioma, and thyroid cancer [22, 24–26]. Moreover, a recent study showed that the prognostic effect of *TERT* promoter mutation was different for each melanoma subtype

[27]. Our study has few samples; hence, it is difficult to be definite about the prognosis. However, we could not conclude that the *TERT* promoter mutation was a poor prognostic factor based on our results.

It would have been indeed useful to analyze telomerase activity and/or *TERT* expression in these tumors. However, fresh-frozen tissues would be required to assess either of them. Unfortunately, materials available for this study were only FFPE specimen from the biopsy, and no frozen tissues were preserved. We could not extract enough RNA from the FFPE tissue samples, and no antibody against *TERT* for immunohistochemistry is currently available. Therefore, it was impossible to examine telomerase activity or *TERT* expression. In most previous reports, *TERT* expression was significantly elevated in cases with unified hotspot mutation compared with the wild-type cases [10, 12–14, 21, 25, 28, 29]. We believe our mutated tumors had elevated *TERT* expression.

Further studies are needed to clarify our findings. We consider two types of prospective cohort studies to investigate the prognostic importance and predictive factor in breast cancer. In one large-scale cohort study, which include prognostic factors of breast cancer such as stage, age, subtype, grade, or other biomarkers with *TERT* promoter mutation, we will be able to investigate whether the *TERT* promoter mutation

is a prognostic factor. On the contrary, a study on ovarian cancer reported that a high *TERT* expression is a predictive factor for the effect of eribulin mesylate [30]. Therefore, another large-scale cohort study may also consider whether the *TERT* promoter mutation is a predictive factor of the effect of an anticancer agent, especially eribulin mesylate.

In conclusion, we have demonstrated the presence of TERT promoter mutation in breast cancer.

Acknowledgments

We thank Nao Nakamura and Rumi Koyama for providing secretarial support during this study. The National Cancer Center Biobank is supported by the Japanese National Cancer Center Research and Development Fund. We thank the individuals whose data and specimens were used for the analyses.

Compliance with ethical standards

Ethical approval and consent to participate

Ethical approval is in accordance with the Declaration of Helsinki.

Funding

This work was supported by funds for Cancer Research and Development [23-B-15] from the Japan Ministry of Health, Labour and Welfare.

Conflicts of interest

The authors have declared no conflicts of interest.

References

1. Siegel R, Miller K, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.

2. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016;534:47–54.

3. Arteaga C. Benefit mixed with caution for buparlisib. Cancer Discov 2017;7:121.

4. Davies BR, Guan N, Logie A, Crafter C, Hanson L, Jacobs V, et al. Tumors with AKT1 E17K mutations are rational targets for single agent or combination therapy with AKT inhibitors. Mol Cancer Ther 2015;14:2441–51.

5. Tamura K, Hashimoto J, Tanabe Y, Kodaira M, Yonemori K, Seto T, et al. Safety and tolerability of AZD5363 in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 2016;77:787–95.

6. Herbert BS, Wright WE, Shay JW. Telomerase and breast cancer. Breast Cancer Res 2001;3:146–9.

7. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science 2013;339:957–9.

8. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT Promoter Mutations in Familial and Sporadic Melanoma. Science 2013;339:959–61.

9. Liu T, Yuan X, Xu D. Cancer-specific telomerase reverse transcriptase (Tert) promoter mutations: Biological and clinical implications. Genes (Basel). 2016;7. pii: E38.

10. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A 2013;110:6021–6.

11. Yoshida M, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, et al. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. Br J Cancer 2015;113:1244-8.

12. Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun 2013;4:2185.

13. Arita H, Narita Y, Fukushima S, Tateishi K, Matsushita Y, Yoshida A, et al. Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. Acta Neuropathol 2013;126:267–76.

14. Arita H, Narita Y, Takami H, Fukushima S, Matsushita Y, Yoshida A, et al. TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. Acta Neuropathol 2013;126:939–41.

15. Liu C, Liu Z, Chen T, Zeng W, Guo Y, Huang T. TERT promoter mutation and its association with clinicopathological features and prognosis of papillary thyroid cancer: a meta-analysis. Sci Rep 2016;6:36990.

16. Nakamura T, Sueoka-Aragane N, Iwanaga K, Sato A, Komiya K, Kobayashi N, et al. Application of a highly sensitive detection system for epidermal growth factor receptor mutations in plasma DNA. J Thorac Oncol 2012;7:1369–81.

17. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod. Pathol. 1998;11:155–68.

18. Wolff AC, Hammond MEH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007;25:118–45.

19. Tsuda H, Akiyama F, Kurosumi M, Sakamoto G, Watanabe T. Establishment of Histological Criteria for High-risk Node-negative Breast Carcinoma for a Multi-institutional Randomized Clinical Trial of Adjuvant Therapy. Jpn J Clin Oncol. 1998;28:486-91.

20. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term

follow-up. Histopathology. 1991;19:403-10.

21. Wu RC, Ayhan A, Maeda D, Kim KR, Clarke BA, Shaw P, et al. Frequent somatic mutations of the telomerase reverse transcriptase promoter in ovarian clear cell carcinoma but not in other major types of gynaecological malignancy. J Pathol. 2014;232:473-81.

22. Tiedje V, Ting S, Herold T, Synoracki S, Latteyer S, Moeller LC, et al. NGS based identification of mutational hotspots for targeted therapy in anaplastic thyroid carcinoma. Oncotarget 2017; 8:42613–20.

23. Zacher A, Kaulich K, Stepanow S, Wolter M, Köhrer K, Felsberg J, et al, Molecular diagnostics of gliomas using next generation sequencing of a glioma-tailored gene panel. Brain Pathol 2017;27:146–59.

24. Griewank KG, Murali R, Puig-Butille JA, Schilling B, Livingstone E, Potrony M, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst 2014;106.

25. Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol 2014;32:2718–26.

26. Simon M, Hosen I, Gousias K, Rachakonda S, Heidenreich B, Gessi M, et al. TERT promoter mutations: A novel independent prognostic factor in primary glioblastomas. Neuro Oncol 2015;17:45–52.

27. Roh MR, Park KH, Chung KY, Shin SJ, Rha SY, Tsao H. Telomerase reverse transcriptase (TERT) promoter mutations in Korean melanoma patients. Am J Cancer Res 2017;7:134-138.

28. Chen Chen, Sheng Han., Lingxuan Meng Zhonghua Li, Xue Zhang, Anhua Wu. TERT Promoter Mutations Lead to High Transcriptional Activity under Hypoxia and Temozolomide Treatment and Predict Poor Prognosis in Gliomas. PLoS One. 2014;9:e100297.

29. Wang N, Liu T, Sofiadis A, Juhlin CC, Zedenius J, Höög A, et al. TERT promoter mutation as an early genetic event activating telomerase in follicular thyroid adenoma (FTA) and atypical FTA. Cancer. 2014;120:2965-79.

30. Yamaguchi S, Maida Y, Yasukawa M, Kato T, Yoshida M, Masutomi K. Eribulin mesylate targets human telomerase reverse transcriptase in ovarian cancer cells. PLoS One. 2014;9:e112438.

Table 1. The characteristics of the three breast cancer patients with *TERT* promoter mutations.

	Case 1	Case 2	Case 3
Age, years	59	41	46
Sex	Female	Female	Female
Initial stage	1A	ND	2A
Histology	IDC	IDC	ILC
Histological grade	2	2	1
Nuclear grade	1	2	1
Estrogen receptor (AS)	Negative (0)	Positive (8)	Positive (8)
Progesterone receptor (AS)	Negative (0)	Positive (8)	Positive (8)
HER2 status (IHC score)	Negative (0)	Negative (0)	Negative (1+)
PIK3CA hotspot mutation	H1047R	Negative	H1047R
TERT hotspot mutation	C250T	C228T	C228T
Relapse-free survival, months	83	226	270
Overall survival, months	100	446	300
Status	Alive	Alive	Alive

ND: no data, IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, AS:

Allred score, HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry.

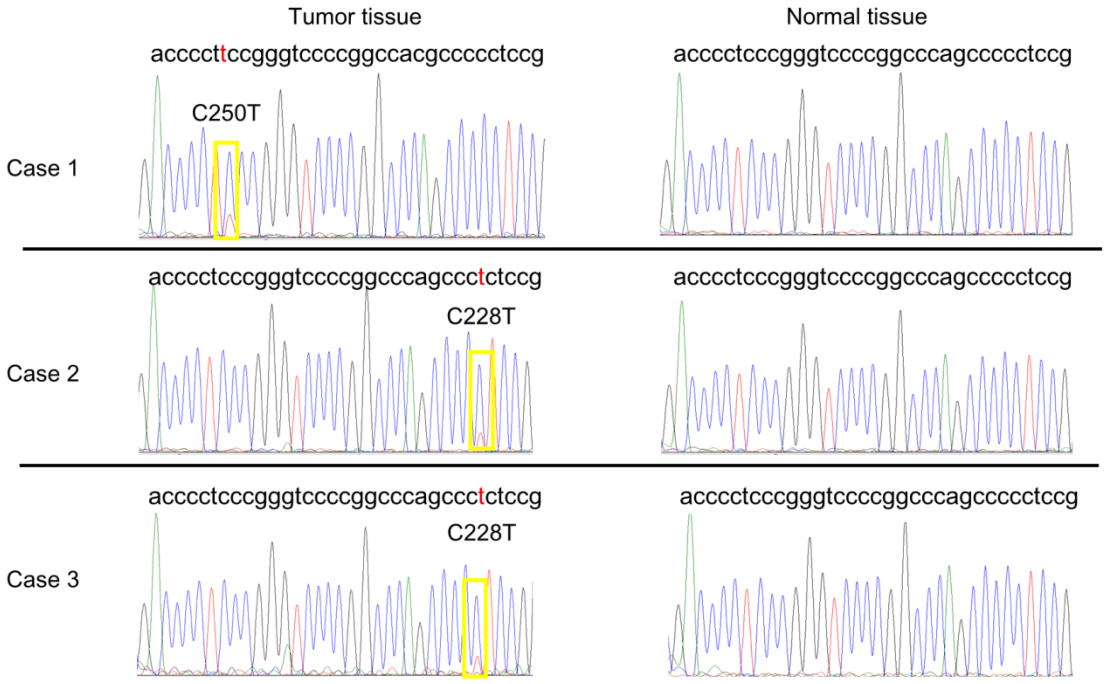


Figure 1

Figure legends

Figure 1. *TERT* promoter mutations in the three breast cancer cases. Sanger sequencing of the tumor tissue DNA revealed C250T (case 1) and C228T (case 2 and 3) somatic mutations in the *TERT* promoter of the three breast cancer patients. All patients demonstrated *TERT* promoter mutations only in their tumor tissue DNA and not in the normal tissue DNA. Positive result for a *TERT* hotspot mutation is indicated by the letter in red font and the corresponding yellow box.

TERT promoter hotspot mutations in breast cancer

Tatsunori Shimoi[1,2], Masayuki Yoshida[3], Yuka Kitamura[1], Tomomi Yoshino[1], Asuka Kawachi[1], Akihiko Shimomura[1], Emi Noguchi[1], Mayu Yunokawa[1], Kan Yonemori[1], Chikako Shimizu[1], Takayuki Kinoshita[4], Koichi Ichimura[5], Takahiro Fukuda[2,6], Yasuhiro Fujiwara[1], Kenji Tamura[1*]

[1] Department of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo, Japan

[2] Course of Advanced Clinical Research of Cancer, Juntendo University, Graduate School of Medicine, Tokyo, Japan

[3] Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tokyo, Japan

[4] Department of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

[5] Division of Brain Tumor Translational Research, National Cancer Center, Tokyo, Japan

[6] Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

*Corresponding author:

Kenji Tamura, MD. PhD.,

Department of Breast and Medical Oncology,

National Cancer Center Hospital,

5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan.

Tel: 81-3-3542-2511;

Fax: 81-3-3542-3815;

Email: ketamura@ncc.go.jp

Abstract

Background Telomerase reverse transcriptase (*TERT*) promoter mutations have been discovered in solid and hematological malignancies, where they reflect *TERT* activation and cell-cycle progression. In melanoma, glioma, and thyroid cancers, *TERT* promoter mutations are associated with a poor prognosis. However, no studies have evaluated the prevalence and prognostic significance of *TERT* promoter mutations in breast cancer.

Methods We analyzed *TERT* promoter hotspot mutations (C228T and C250T) using direct sequencing of DNA from 319 tumor tissues. We also collected clinical data from cases that were positive for *TERT* promoter mutations.

Results We detected *TERT* promoter mutations in three (0.9%) of the 319 cases. Two patients had hormone receptor-positive and human epidermal growth factor receptor 2-negative cancer, while the third patient had triple-negative cancer. All three patients had initially been diagnosed with operable breast cancer and undergone surgical treatment. The relapse-free survivals of these patients were 83, 226, and 270 months, respectively. The mutations were C250T in the triple-negative cancer case and C228T in the remaining two cases.

Conclusion Given the rarity of *TERT* promoter mutations, further studies are needed to confirm their prognostic significance in breast cancer cases.

Keywords: Breast cancer, telomerase reverse transcriptase, *TERT* promoter mutation,

Introduction

Breast cancer is a common cancer and the leading global cause of cancer-related mortality among women [1]. In addition, breast cancer is a heterogeneous condition that is categorized into four subtypes according to pathological review, hormone receptor status, and human epidermal growth factor receptor 2 (HER2) status. Furthermore, using whole-genome sequencing, numerous somatic and driver mutations have been identified in breast cancer [2]. This information has allowed physicians to stratify patients according to their tumor's molecular characteristics and select appropriate therapies. Recent clinical trials have indicated that mutations in the genes for phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) and RAC-alpha serine/threonine-protein kinase (*AKT1*) may predict the response to PI3K and AKT inhibitors, respectively [3–5].

Telomerase is a DNA polymerase that maintains the length of telomeres at the end of chromosomes. Its activity is relatively high in stem cells and is downregulated in normal somatic cells. However, genetic mutations can also affect the non-coding regulatory region of the telomerase reverse transcriptase (*TERT*) gene's promoter, and telomerase can be activated by mutations in the *TERT* promoter. Many malignant tumor cells have TERT expression or telomerase activity, with >90% of breast cancer cases

having telomerase activity [6].

In 2013, somatic hotspot mutations in the promoter region of *TERT* were reported among patients with melanoma [7,8]. In addition to these two common hotspots identified on chromosome 5: 1,295,228 C>T (C228T) and 1,295,250 C>T (C250T), the tandem mutations of 1,295,228/1,295,229 CC>TT (C228T/C229T) and 1,295,242/1,295,243 CC>TT (C242T/C243T) have also been reported in melanoma with *TERT* promoter mutation. In a previous report, mutation frequencies for C228T, C250T, C228T/C229T and C242T/C243T in melanoma cell lines were 46/168 (27%), 64/168 (38%), 7/168 (4.2%) and 8/168 (4.8%), respectively. On the contrary, no mutation sites other than C228T and C250T have been reported in glioma. In one large retrospective cohort, the mutation frequencies of C228T and C250T in primary glioblastoma with *TERT* promoter mutation were 123/256 (48%) and 56/256 (22%), respectively [13]. Since the first report of melanoma, >30 types of tumors have been found to contain *TERT* promoter mutations, hepatocellular carcinoma (41%), thyroid cancer (11–43%), ovarian clear cell carcinoma (16%), bladder cancer (63%), and phyllodes tumors of the breast (65%) [9,10,11]. In these types of cancer other than melanoma, C228T and C250T were also hotspots of *TERT* promoter mutations, in which C228T is more dominant than C250T. These hotspot mutations are associated

with a poor prognosis [7,12,14–15]. However, to the best of our knowledge, no reports have evaluated the prevalence and prognostic significance of *TERT* promoter mutations in human breast cancer. Therefore, the present study aimed to provide information regarding *TERT* promoter mutations in samples from human breast cancer.

Materials and Methods

We retrospectively evaluated 319 breast cancer specimens (125 frozen specimens and 194 formalin-fixed paraffin-embedded [FFPE] specimens) that were provided by the National Cancer Center Biobank of Japan and had been obtained between January 1983 and November 2015. Three patients had metachronous breast cancer and we included all of their samples in this study. We extracted DNA from the frozen specimens using the QIAamp DNA FFPE tissue kit (QIAGEN, Tokyo) and from the FFPE specimens using the QIAamp DNA Mini Kit (QIAGEN, Tokyo), according to the manufacturer's recommendations. The *TERT* promoter mutations (C228T and C250T) were identified using direct sequencing according to the previously reported methods [12,13], and the direct sequencing was performed by FASMAC (Tokyo, Japan). The forward primer's sequence was 5'-gtaaaacgacggccagcaggaaacagctatgacccagctccgcctcctccg-3' and the reverse primer's

sequence was 5'- gctgcctgaaactcgcgcc-3'. Mutations in the *PIK3CA* gene (E542K, E545K, or H1047R) were detected using quenching probe system by the i-densy IS-5320 system (ARKRAY Inc., Kyoto, Japan) [16].

Estrogen receptor (ER) and progesterone receptor (PgR) were classified positive if ≥10% of tumor cells demonstrated positive nuclear staining for ER or PgR, respectively [17]. HER2 positivity was classified according to the recommendation of the American Society of Clinical Oncology/College of American Pathologists guidelines [18]. Hormone receptor positive was defined as positive for ER or PgR. Histological and nuclear grades were reported according to previously reported criteria [19, 20].

Relapse-free survival was defined as the time between the day of diagnosis and disease progression or last follow-up. Overall survival was defineed as the time between the day of diagnosis and the day of death or last follow-up.

This study's retrospective protocol was approved by the National Cancer Center Institutional Review Board (No. 2014-092). Written informed consent was not obtained from the patients. The results of this study have been published on our hospital's web page.

Results

Sequencing was successful for all 319 specimens, and we detected *TERT* promoter mutations in three samples (0.9%). The *TERT* promoter mutations were only detected in the tumor tissue DNA and were not detected in the three patients' normal tissue DNA. The three patients' characteristics are shown in Table 1. All patients were women who had experienced a relatively late relapse, after undergoing surgery because of an early diagnosis of breast cancer. Two patients demonstrated relapse-free survivals of >200 months. The histological results were invasive ductal carcinoma in two cases and invasive lobular carcinoma in one case. Two patients had hormone receptor-positive and HER2-negative breast cancer, while one patient had triple-negative breast cancer (TNBC). C250T mutations were noted in the TNBC case and C228T in the two hormone receptor-positive cases (Figure 1). Two of these patients had mutations in the *PIK3CA* kinase domain (H1047R) (Table 1).

Discussion

This is the first detailed report regarding *TERT* promoter mutations in human breast cancer, and we identified *TERT* promoter mutations in three (0.9%) of 319

samples. However, a previous report identified two cases with *TERT* promoter mutations among eight human breast cancer cell lines (25%) [12]. Nevertheless, another report failed to detect *TERT* promoter hotspot mutations in 88 breast cancer samples [10]. In the present study, we found that two of the three samples with *TERT* promoter mutations also had *PIK3CA* kinase domain mutations. In ovarian cancer, *TERT* promoter mutation and *PIK3CA* mutation are considered mutually exclusive [21], whereas this coexistence is reported in other carcinomas [22–23]. Given that *PIK3CA* mutation is a relatively common mutation in breast cancer, we investigated the coexistence of these mutations. Interestingly, *PIK3CA* mutations only exist in approximately 30% of patients with hormone receptor-positive breast cancer and in approximately 10–20% of TNBC cases. Moreover, *TERT* promoter mutations coexist with *PIK3CA* mutations in 12% of anaplastic thyroid cancers [22], but only in 7.5% of gliomas [23]. Thus, it is possible that a relationship exists between *TERT* promoter and *PIK3CA* kinase domain mutations.

TERT promoter mutations occur during the early stage of glioma or thyroid cancers and are associated with a poor prognosis among patients with melanoma, glioma, and thyroid cancer [22, 24–26]. Moreover, a recent study showed that the prognostic effect of *TERT* promoter mutation was different for each melanoma subtype

[27]. Our study has few samples; hence, it is difficult to be definite about the prognosis. However, we could not conclude that the *TERT* promoter mutation was a poor prognostic factor based on our results.

It would have been indeed useful to analyze telomerase activity and/or *TERT* expression in these tumors. However, fresh-frozen tissues would be required to assess either of them. Unfortunately, materials available for this study were only FFPE specimen from the biopsy, and no frozen tissues were preserved. We could not extract enough RNA from the FFPE tissue samples, and no antibody against *TERT* for immunohistochemistry is currently available. Therefore, it was impossible to examine telomerase activity or *TERT* expression. In most previous reports, *TERT* expression was significantly elevated in cases with unified hotspot mutation compared with the wild-type cases [10, 12–14, 21, 25, 28, 29]. We believe our mutated tumors had elevated *TERT* expression.

Further studies are needed to clarify our findings. We consider two types of prospective cohort studies to investigate the prognostic importance and predictive factor in breast cancer. In one large-scale cohort study, which include prognostic factors of breast cancer such as stage, age, subtype, grade, or other biomarkers with *TERT* promoter mutation, we will be able to investigate whether the *TERT* promoter mutation

is a prognostic factor. On the contrary, a study on ovarian cancer reported that a high *TERT* expression is a predictive factor for the effect of eribulin mesylate [30]. Therefore, another large-scale cohort study may also consider whether the *TERT* promoter mutation is a predictive factor of the effect of an anticancer agent, especially eribulin mesylate.

In conclusion, we have demonstrated the presence of TERT promoter mutation in breast cancer.

Acknowledgments

We thank Nao Nakamura and Rumi Koyama for providing secretarial support during this study. The National Cancer Center Biobank is supported by the Japanese National Cancer Center Research and Development Fund. We thank the individuals whose data and specimens were used for the analyses.

Compliance with ethical standards

Ethical approval and consent to participate

Ethical approval is in accordance with the Declaration of Helsinki.

Funding

This work was supported by funds for Cancer Research and Development [23-B-15] from the Japan Ministry of Health, Labour and Welfare.

Conflicts of interest

The authors have declared no conflicts of interest.

References

1. Siegel R, Miller K, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.

2. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016;534:47–54.

3. Arteaga C. Benefit mixed with caution for buparlisib. Cancer Discov 2017;7:121.

4. Davies BR, Guan N, Logie A, Crafter C, Hanson L, Jacobs V, et al. Tumors with AKT1 E17K mutations are rational targets for single agent or combination therapy with AKT inhibitors. Mol Cancer Ther 2015;14:2441–51.

5. Tamura K, Hashimoto J, Tanabe Y, Kodaira M, Yonemori K, Seto T, et al. Safety and tolerability of AZD5363 in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 2016;77:787–95.

6. Herbert BS, Wright WE, Shay JW. Telomerase and breast cancer. Breast Cancer Res 2001;3:146–9.

7. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science 2013;339:957–9.

8. Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, et al. TERT Promoter Mutations in Familial and Sporadic Melanoma. Science 2013;339:959–61.

9. Liu T, Yuan X, Xu D. Cancer-specific telomerase reverse transcriptase (Tert) promoter mutations: Biological and clinical implications. Genes (Basel). 2016;7. pii: E38.

10. Killela PJ, Reitman ZJ, Jiao Y, Bettegowda C, Agrawal N, Diaz LA Jr, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. Proc Natl Acad Sci U S A 2013;110:6021–6.

11. Yoshida M, Ogawa R, Yoshida H, Maeshima A, Kanai Y, Kinoshita T, et al. TERT promoter mutations are frequent and show association with MED12 mutations in phyllodes tumors of the breast. Br J Cancer 2015;113:1244-8.

12. Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun 2013;4:2185.

13. Arita H, Narita Y, Fukushima S, Tateishi K, Matsushita Y, Yoshida A, et al. Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. Acta Neuropathol 2013;126:267–76.

14. Arita H, Narita Y, Takami H, Fukushima S, Matsushita Y, Yoshida A, et al. TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. Acta Neuropathol 2013;126:939–41.

15. Liu C, Liu Z, Chen T, Zeng W, Guo Y, Huang T. TERT promoter mutation and its association with clinicopathological features and prognosis of papillary thyroid cancer: a meta-analysis. Sci Rep 2016;6:36990.

16. Nakamura T, Sueoka-Aragane N, Iwanaga K, Sato A, Komiya K, Kobayashi N, et al. Application of a highly sensitive detection system for epidermal growth factor receptor mutations in plasma DNA. J Thorac Oncol 2012;7:1369–81.

17. Allred DC, Harvey JM, Berardo M, Clark GM. Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod. Pathol. 1998;11:155–68.

18. Wolff AC, Hammond MEH, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol. 2007;25:118–45.

19. Tsuda H, Akiyama F, Kurosumi M, Sakamoto G, Watanabe T. Establishment of Histological Criteria for High-risk Node-negative Breast Carcinoma for a Multi-institutional Randomized Clinical Trial of Adjuvant Therapy. Jpn J Clin Oncol. 1998;28:486-91.

20. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term

follow-up. Histopathology. 1991;19:403-10.

21. Wu RC, Ayhan A, Maeda D, Kim KR, Clarke BA, Shaw P, et al. Frequent somatic mutations of the telomerase reverse transcriptase promoter in ovarian clear cell carcinoma but not in other major types of gynaecological malignancy. J Pathol. 2014;232:473-81.

22. Tiedje V, Ting S, Herold T, Synoracki S, Latteyer S, Moeller LC, et al. NGS based identification of mutational hotspots for targeted therapy in anaplastic thyroid carcinoma. Oncotarget 2017; 8:42613–20.

23. Zacher A, Kaulich K, Stepanow S, Wolter M, Köhrer K, Felsberg J, et al, Molecular diagnostics of gliomas using next generation sequencing of a glioma-tailored gene panel. Brain Pathol 2017;27:146–59.

24. Griewank KG, Murali R, Puig-Butille JA, Schilling B, Livingstone E, Potrony M, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst 2014;106.

25. Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol 2014;32:2718–26.

26. Simon M, Hosen I, Gousias K, Rachakonda S, Heidenreich B, Gessi M, et al. TERT promoter mutations: A novel independent prognostic factor in primary glioblastomas. Neuro Oncol 2015;17:45–52.

27. Roh MR, Park KH, Chung KY, Shin SJ, Rha SY, Tsao H. Telomerase reverse transcriptase (TERT) promoter mutations in Korean melanoma patients. Am J Cancer Res 2017;7:134-138.

28. Chen Chen, Sheng Han., Lingxuan Meng Zhonghua Li, Xue Zhang, Anhua Wu. TERT Promoter Mutations Lead to High Transcriptional Activity under Hypoxia and Temozolomide Treatment and Predict Poor Prognosis in Gliomas. PLoS One. 2014;9:e100297.

29. Wang N, Liu T, Sofiadis A, Juhlin CC, Zedenius J, Höög A, et al. TERT promoter mutation as an early genetic event activating telomerase in follicular thyroid adenoma (FTA) and atypical FTA. Cancer. 2014;120:2965-79.

30. Yamaguchi S, Maida Y, Yasukawa M, Kato T, Yoshida M, Masutomi K. Eribulin mesylate targets human telomerase reverse transcriptase in ovarian cancer cells. PLoS One. 2014;9:e112438.

Table 1. The characteristics of the three breast cancer patients with *TERT* promoter mutations.

	Case 1	Case 2	Case 3
Age, years	59	41	46
Sex	Female	Female	Female
Initial stage	1A	ND	2A
Histology	IDC	IDC	ILC
Histological grade	2	2	1
Nuclear grade	1	2	1
Estrogen receptor (AS)	Negative (0)	Positive (8)	Positive (8)
Progesterone receptor (AS)	Negative (0)	Positive (8)	Positive (8)
HER2 status (IHC score)	Negative (0)	Negative (0)	Negative (1+)
PIK3CA hotspot mutation	H1047R	Negative	H1047R
TERT hotspot mutation	C250T	C228T	C228T
Relapse-free survival, months	83	226	270
Overall survival, months	100	446	300
Status	Alive	Alive	Alive

ND: no data, IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma, AS:

Allred score, HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry.

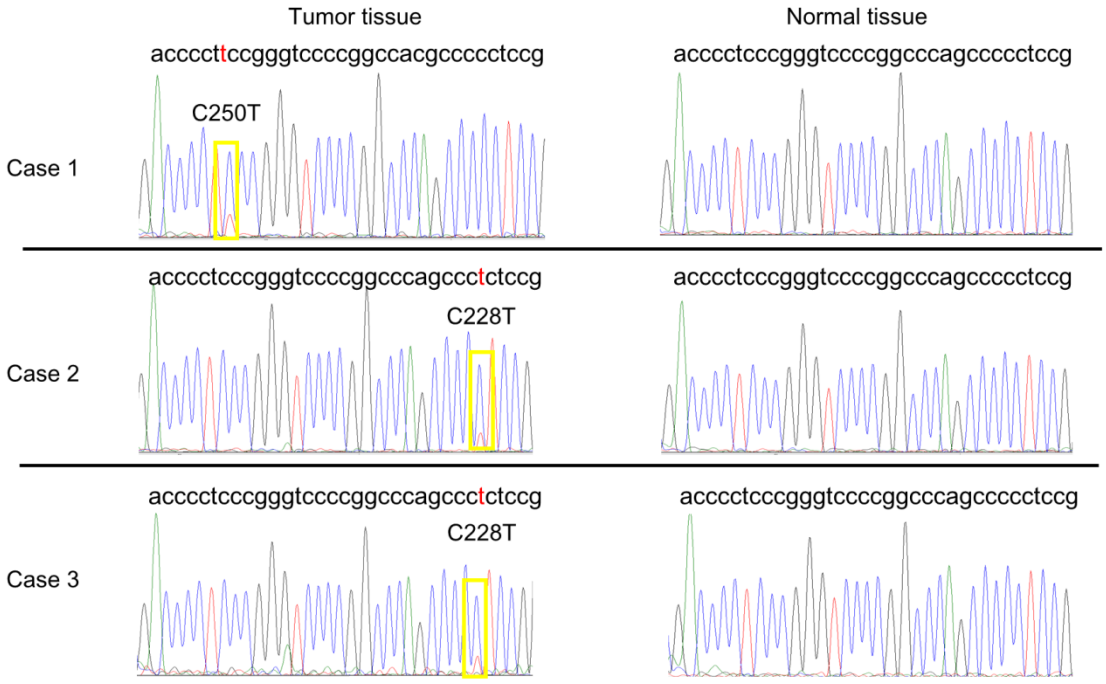


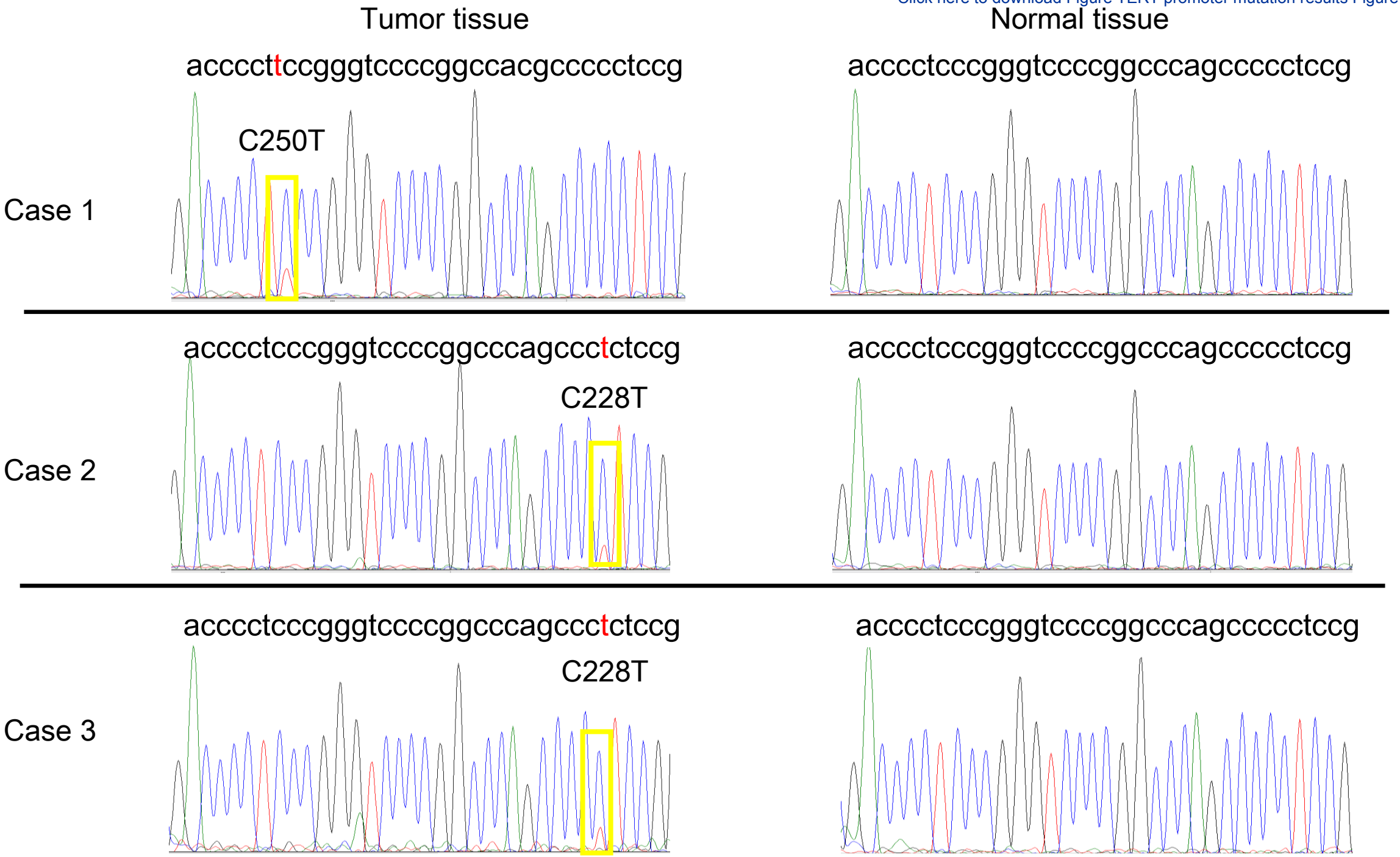
Figure 1

Figure legends

Figure 1. *TERT* promoter mutations in the three breast cancer cases. Sanger sequencing of the tumor tissue DNA revealed C250T (case 1) and C228T (case 2 and 3) somatic mutations in the *TERT* promoter of the three breast cancer patients. All patients demonstrated *TERT* promoter mutations only in their tumor tissue DNA and not in the normal tissue DNA. Positive result for a *TERT* hotspot mutation is indicated by the letter in red font and the corresponding yellow box.

Figure

Click here to download Figure TERT promoter mutation results Figure 1_.pptx



Breast Cancer

CERTIFICATION FOR MANUSCRIPT SUBMISSION

Manuscript No.

Title: *TERT* promoter hotspot mutations in breast cancer

I/We hereby certify to the editor of Breast Cancer that our manuscript meets all the following conditions:

1. None of the material of the manuscript has been published elsewhere in whole or in part, except in abstract form.

2. The manuscript is not simultaneously under consideration for publication elsewhere in a journal, a book, or electronic media.

3. All authors have agreed to be so listed and have seen and approved the manuscript, its contents, and its submission to Breast Cancer.

4. Written permission from the copyright holder(s) of any table(s) and/or figure(s) cited in the manuscript has been obtained, and citation is indicated in the manuscript.

Author's name(block letters):	Signature:	Date:
KENJI TAMURA		Sep 5. 2017
CHIKAKO SHIMIZU	C.Shimizu	Sep 5. 2017
KAN YONEMORI		Sep 5, 2017
Akihiko Shimomura		Sep 5, 2017
Emi Noguchi	Emi Noguchi	Sep 6, 2017
YASUHIRO FUJIWARA		Sep 6, 2017
ASUKA KAWACHI	Asuka Kawachi	Sep 16. 2017
TAKAYUKI KINOSHITA	Takayuki Kinoshita	Sep 13. 2017
FUKUDA, TAKAHIRO		Sep 15, 2017
MASAYUKI YOSHIDA	Masayuki Yoshida	Sep. 14, 2017

Manuscript No.

Title: *TERT* promoter hotspot mutations in breast cancer

Author's name(block letters):	Signature:	Date:
KOICHI ICHIMURA	Koichi Ichimura	Sep. 26, 2017
MAYU YUNOKAWA	Mayu Yunokawa	Sep 27, 2017
TOMOMI YOSHINO	Tomomi Yoshino	Sep. 27, 2017
YUKA KITAMURA	Yuka Kitamura	Sep. 27, 2017
TATSUNORI SHIMOI	Tatsunori Shimoi	Sep. 27, 2017

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Kenji Tamura

Signature: Kenji Tamura Date: Sep 5, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Chikako Shimizu

Signature: C. Shimizu Date: Sep 5. 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

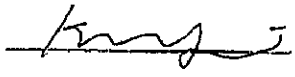
When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Kan Yonemori

Signature:  Date: Sep 5, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Akihiko Shimomura

Signature: Akihiko Shimomura Date: Sep 5, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Emi Noguchi

Signature: Emi Noguchi Date: Sep 6, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Yasuhiro Fujiwara

Signature: Yasuhiro Fujiwara Date: Sep 6, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Asuka Kawachi

Signature: Asuka Kawachi Date: Sep 16, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Takayuki Kinoshita

Signature: Takayuki Kinoshita Date: Sep 13. 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Takahiro Fukuda

Signature: _____ Date: Sep 15, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Masayuki Yoshida

Signature: Masayuki Yoshida Date: Sep. 14, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Koichi Ichimura

Signature: _____ Date: Sep. 26, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Mayu Yunokawa

Signature: Mayu Yunokawa Date: Sep 27, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Tomomi Yoshino

Signature: Tomomi Yoshino Date: Sep. 27, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Yuka Kitamura

Signature: Yuka Kitamura Date: Sep. 27, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.

[Breast Cancer] **Conflict of Interest Disclosure Statement** (Form 2)

Manuscript No.: _____

Manuscript Title: ***TERT* promoter hotspot mutations in breast cancer**

Each author is required to complete and return this form to the corresponding author.

(Please do not send this to the JBCS office.)

When submitting a manuscript to *Breast Cancer*, all authors are required to disclose any financial relationship within the last three years with a biotechnology manufacturer, a pharmaceutical company, or other commercial entity that has an interest in the subject matter or materials discussed in the manuscript. When your manuscript is accepted for publication, the disclosures will appear in your article as a "Conflict of Interest Statement" in *Breast Cancer* as follows.

Conflict of Interest Statement: A (author name) serves as a consultant to Z (entity name); B's spouse is chairman of Y; C received a research grant from X; D received lecture fees from V; E holds a patent on U; F has been reimbursed by T for attending several conferences; G received honoraria for writing promotional material for S; H has no conflict of interest.

If you, your spouse, a relative in the first degree, or any other person who shares his or her income or assets has any of the listed relationships with a commercial entity that has an interest in the subject matter in your manuscript, please refer to the JBCS's definition of conflict of interest which must be disclosed. Next, check the appropriate "Yes" box below and provide details. If the listed relationship does not apply to you or your family member, check the appropriate "No" box.

1. Position as officer or advisor of company or for-profit organization and remuneration amount	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization.
	Company or organization name: Position: Amount of remuneration:
2. Stock ownership and profit resulting from the stock	Applicable / Not applicable (Circle one) If applicable, record the following details for each company.
	Company name: Number of shares: Declared share price: Profit from the share:
3. Remuneration paid as patent royalties or licensing fees from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record details for each patent.
	Company or organization name: Patent name: Royalties or licensing fees:
4. Honoraria (such as lecture fees) paid by company or for-profit organization as compensation	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of lecture fees:
5. Manuscript fees paid from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or funding organization.
	Company or organization name: Amount of manuscript fees:
6. Research funding from companies or for-profit organizations	Applicable / Not applicable (Circle one) If applicable, record the following details for each clinical research project.
	Company or organization name: Name of clinical research: Amount of remuneration:
7. Other remunerations (travel, gifts, or in-kind payments not directory related to research)	Applicable / Not applicable (Circle one) If applicable, record the following details for each company or organization name.
	Company or organization name: Amount of remuneration:

Print name: Tatsunori Shimoi

Signature: Tatsunori Shimoi Date: Sep. 27, 2017

All forms from each author must be uploaded online and submitted with the manuscript at the time of submission by the corresponding author.