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Resistance to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer: Importance of Ki-67 Labeling Index and the Recognition of Apocrine-type Lesions

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Abstract

Triple-Negative Breast Cancer (TNBC) encompasses heterogeneous subtypes. Apocrine-type TNBC, defined as TNBC immunoreactive for both androgen receptor and forkhead-box protein A1, should be distinguished from basal-like TNBC. In apocrine-type TNBC, neoadjuvant chemotherapy (NAC) tends to be ineffective, but with a favorable prognosis despite chemoresistance. We analyzed 51 cases of TNBC in stages I and II. Thirty-four TNBCs treated with NAC were divided into "good responders" (n=22) showing therapeutic effect G2b or G3 in surgical specimens and "poor responders" (n=12) with therapeutic effect G0, G1a, G1b and G2a. NAC was spared in 17 cases (categorized as the non-NAC group). TNBC other than apocrine-type (n=16) and special types (myoepithelial, medullary, adenoid cystic and spindle cell carcinomas, n=6) was categorized as basal-like subtype (n=29). The prognosis was evaluated in each category. NAC showed significant effects against basal-like TNBC with high Ki-67 labeling (≥ 50%), and tumor-infiltrating lymphocytes predicted high chemosensitivity. NAC was ineffective and avoidable in TNBC of apocrine- and special types showing low (<50%) Ki-67 labeling. Ten (59%) lesions in the non-NAC group belonged to the apocrine-type. When clinical complete remission shown by contrast-enhanced magnetic resonance imaging was reached in the course of NAC against basal-like TNBC, the NAC period was shortened (de-escalated) in 14 (64%) of 22 good responders. Disease-free and overall survival was excellent in all groups. The following two hypothetical proposals should be proven by large-scale clinical trials. Immunohistochemical recognition of apocrine-type TNBC with low Ki-67 labeling is important for avoiding ineffective/unnecessary NAC. By employing appropriate clinical imaging, de-escalation of NAC is achievable in basal-like TNBC with high Ki-67 labeling.

Keywords: Androgen receptor • Apocrine-type • Avoidance of chemotherapy • Basal-like subtype • De-escalation of chemotherapy • FOXA1 • Ki-67 • Triple-negative breast cancer

Abbreviations

AR: Androgen Receptor; cCR: Clinical Complete Remission; CK5/6: Cytokeratin 5/6; CK14: Cytokeratin 14; CMF: Cyclophosphamide/Methotrexate/5-Fluorouracil; DFS: Disease-Free Survival; DTX: Docetaxel; EC: Epirubicin/Cyclophosphamide; EGFR: Epidermal Growth Factor Receptor; ER: Estrogen Receptor; FOXA1: Forkhead-box Protein A1; GCDFP15: Gross Cystic Disease Fluid Protein 15; HE: Hematoxylin and Eosin; HER2: Human Epidermal growth factor Receptor type 2; LAR: Luminal Androgen Receptor; MRI: Magnetic Resonance Imaging; nabPTX: Nanoparticle albumin-bound paclitaxel; NAC: Neoadjuvant Chemotherapy; OS: Overall Survival; pCR: Pathological Complete Remission; PD: Progressive Disease; PgR: Progesterone Receptor; PTX: Paclitaxel; TILs: Tumor-Infiltrating Lymphocytes; TNBC: Triple-Negative Breast Cancer

Introduction

Breast cancer is the most common women's malignancy in the world [1]. The Center for Cancer Control Information Services of National Cancer Center, Tokyo, Japan, estimated 94,400 new cases of breast cancer with 15,700 deaths in 2021 in Japan, and every one of 11 Japanese women suffer breast cancer during the lifetime [2].

Based on the profiling pattern of mRNA expression, invasive breast cancer has been classified into biologically and clinically distinct intrinsic subtypes. The basal-like subtype, microscopically characterized by poor tubule formation, high histological grading and high mitotic activity, tends to be seen in a younger age group, and accompanies frequent BRCA mutation, early disease recurrence and poor clinical outcome [3-6]. In the daily clinical practice, each intrinsic subtype can be predicted by immunostaining using formalin-fixed, paraffin-embedded sections [7-9]. The basal-like subtype is immunohistochemically negative for Estrogen Receptor (ER), Progesterone Receptor (PgR) and Human Epidermal growth factor Receptor type 2 (HER2), representing Triple-Negative Breast Cancer (TNBC).

TNBC occupies 15% of breast cancer. Cytotoxic chemotherapy (neoadjuvant chemotherapy: NAC) should be used for TNBC of basal-like subtype [10,11]. However, NAC is poorly effective in more than half of TNBC cases, whereas some cases of TNBC show a favorable prognosis despite chemoresistance [12-14]. Figure 1 displays data of the rates of response to NAC according to the breast cancer subtypes in the period from 2003 to 2015 (n=196) in Yokohama Breast and Gastrointestinal Clinic, Yokohama, Japan [15].

In 2012, one of the authors Tsutsumi defined apocrine-type breast cancer as an immunohistochemically ER/PgR-negative and Androgen Receptor (AR)-positive lesion, occupying 44 (13.5%) out of 325 invasive ductal carcinomas [16]. Classical apocrine appearance in Hematoxylin and Eosin (HE) staining was microscopically discerned only in half of these apocrine-type lesions, particularly in those with low histological grading. HER2 overexpression was seen in half of the apocrine-type breast cancer (23/44: 52%). Namely, the remaining half of the apocrine-type breast

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cancers lacked HER2 overexpression, and thus were categorized in TNBC. When compared with basal-like TNBC which is quadruple-negative for ER, PgR, AR and HER2, the apocrine-type TNBC revealed lower histological grading and lower Ki-67 labeling [16]. Patients with apocrine-type TNBC have a better prognosis than those with basal-like TNBC, despite lower pathological Complete Response (pCR) rates after NAC [17-23].

In 2020, Kubouchi et al. re-defined the apocrine-type breast cancer as an ER/PgR-negative and AR/forkhead-box protein A1 (FOXA1)-positive lesion [24], as has been indicated by several investigators [25,26]. FOXA1, also called hepatocyte nuclear factor 3α, is a member of intranuclear transcription factors of the forkhead gene family [27]. In normal and cancerous breast and prostate, FOXA1 co-localizes with ER or AR in the nuclei, and enhances interaction of the hormone receptor with chromatin [28-32].

Molecular transcriptomic analyses have indicated the existence of the molecular apocrine subgroup [33-35]. The molecular apocrine lesion shows AR pathway activation with negativity of ER and PgR and positivity of AR and FOXA1, and in 50% of these cases, HER2 is overexpressed, fully in line with our pervious descriptions [16, 24]. Recent molecular studies indicated Luminal Androgen Receptor (LAR) subgroup [36-38], which corresponds to the HER2-negative molecular apocrine breast cancer [33].

Tumor-Infiltrating Lymphocytes (TILs), particularly rich in TNBC, are a microscopic predictor predicting excellent responses to NAC, and TILs-rich TNBC may thus molecularly represent TNBC of immunomodulatory intrinsic subtype [39-42]. Apocrine-type TNBC occasionally accompanies the lymphoid stroma [43]. In the present review, we evaluated effects of NAC against TNBC in stages I and II and the clinical outcome of both the basal-like (quadruple-negative) type and apocrine-type by analyzing a total of 51 TNBC cases, including 34 with NAC (NAC group) and 17 without NAC (non-NAC group), as has already been reported [24]. As NAC, the Anthracycline (A)-based regimen was followed by the Taxane (T)-based regimen. The standardized cycle numbers were 4 cycles for A and 12 cycles for T, as well as 3 for A and 15 for T or 6 for A and 6 for T. When the tumor size was significantly regressed by palpation and on ultrasound examination, the chelated non-ionic gadolinium-enhanced Magnetic Resonance Imaging (MRI) analysis was performed with an ad libitum fashion to evaluate significant tumor regression. Surgical procedures were performed thereafter to confirm pathological therapeutic effects. It should be emphasized that we can achieve a) the avoidance of NAC for the apocrine-type TNBC with low Ki-67 labeling and b) period-shortening (de-escalation) of NAC for basal-like TNBC with high Ki-67 labeling. The both facts allow patients to relieve or avoid excessive adverse drug reactions.

Patients

In the period from 2011 to 2016, a total of 434 patients with operable primary breast cancer, including 48 patients with ductal carcinoma *in situ*, and 386 with operable invasive breast cancer, were experienced in Yokohama Breast and Gastrointestinal Clinic, Yokohama, Japan. The breast cancer subtypes were judged by immunohistochemical findings of the biopsy specimen [7-9,16]. NAC was administered to a total of 122 patients, and the rest of the patients received surgery without NAC. Sixty-two patients had operable TNBC, including 43 TNBC patients with NAC and 19 TNBC patients without NAC. Thirty-four (79%) of 43 patients with NAC were clinically categorized in stages I and II. Among 19 TNBC patients without NAC, 17 (89%) patients were categorized in stages I and II. Surgical procedures were performed in Kawasaki Municipal Ida Hospital, Nakahara-ku, Kawasaki, and Saiseikai Yokohama-shi Tobu Hospital, Tsurumi-ku, Yokohama.

Table 1 summarizes the age of the patients, the tumor size and the mean follow-up period of the NAC group (n=34), including basal-like TNBC (n=26), apocrine-type TNBC (n=6) and special-type TNBC (n=2), as well as the non-NAC group (n=17).

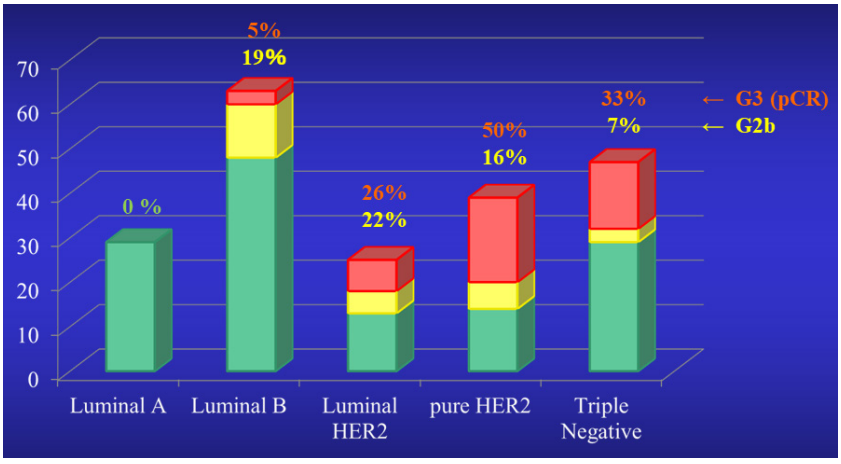

Figure 1. Effects of NAC against breast cancer subtypes (luminal A, luminal B, luminal HER2, pure HER2 and TNBC). Analysis of 196 cases in Yokohama Breast and Gastrointestinal Clinic in the period from 2003 to 2015. The ratios of the good responder (G2b and G3) are demonstrated in red plus yellow color. NAC was relatively effective to HER2 types (luminal HER2 and pure HER2). The response rate of TNBC was only 40%. **Note:** (■) 0.1a.1b.2a; (■) G2b; (■) G3 (pCR)

Table 1. Clinical data of cases of TNBC in stages I and II. Footnote; TNBC: Triple-Negative Breast Cancer.

Patient group	n	Age (year) (range, mean/median)	Tumor size (mm) (range, mean/median)	Follow-up period (mo) (range, mean/median)
NAC group	34	34-71, 54.0/53.5	6-31, 18.3/18	29-104, 64.9/66
Basal-like TNBC	26	34-71, 54.3/53.5	8-31, 19.4/19.5	30-104, 65.9/70
Apocrine-type TNBC	6	38-71, 57.3/62	14-24, 17.5/17	29-101, 68.7/70
Special-type TNBC	2	40-41, 40.5/40.5	6-7, 6.5/6.5	35-44, 39.5/39.5
Non-NAC group	17	37-87, 61.3/60	6-40, 17.5/18	19-129, 75.1/72

Evaluation of Effects of NAC

Reportedly, NAC and adjuvant chemotherapy reveal comparable effects on operable breast cancer [44-46]. NAC has an advantage to allow an opportunity to observe tumor regression by palpation or on image, enabling rapid assessment of clinical response [47]. NAC reveals favorable effects on down-staging of cancer, availability of conservative surgery and increased DFS and OS [12-14,47,48]. NAC has induced pCR in roughly one third of TNBC cases [13,14], and in pCR-induced TNBC cases, excellent (90%) long-term survival was obtained [12,49]. NAC was particularly effective in 52% of basal-like TNBC, whereas low effectiveness (10%) of NAC was seen in LAR-type TNBC [50]. Similar findings have been reported by the Japanese groups [51-53].

As NAC, the Anthracycline (A)-based regimen (epirubicin and cyclophosphamide with or without 5-fluorouracil, 3 weeks/cycle) followed by the Taxane (T)-based regimen (weekly paclitaxel (PTX), triweekly docetaxel (DTX) or weekly nanoparticle albumin-bound paclitaxel (nabPTX)) was administered, in accordance with the National Comprehensive Cancer Network Guidelines [54] and the Guidelines for Breast Cancer in Japan [55]. The standardized cycle numbers were 4 cycles for A and 12 cycles for T, as well as 3 for A and 15 for T or 6 for A and 6 for T. Triweekly DTX was comparable with three cycles of weekly PTX or weekly nabPTX. When the tumor size was significantly regressed down to 5 mm or less on ultrasound examination, the chelated non-ionic gadolinium-enhanced Magnetic Resonance Imaging (MRI) analysis was performed with an ad libitum fashion. We did not accept systematic MRI formulation for the NAC response. By palpation, the tumor commonly becomes unpalpable when the tumor size is shrunken to less than 10 mm. By ultrasound screening, tumor regressed to 5 mm in diameter (or 50 mm^3 in volume) is recognizable. The period of NAC became shorter than the standardized cycles, and we regarded such situation as period-shortening or de-escalation of NAC. As surgical procedures, partial mastectomy (breast-conserving surgery) with post-operative whole breast irradiation or total mastectomy, including nipple-sparing mastectomy without reconstruction, was performed in the respective cases. In one case in the good responder, radiotherapy was omitted after partial mastectomy because of patient's refusal. Four cases with BRCA mutations (all categorized in the good responder) underwent total mastectomy with breast reconstruction.

Disease-Free Survival (DFS) was defined as the interval between the date of biopsy confirmation of the primary cancer and the date at which relapse was confirmed or the date of the last follow-up, as of the end of March, 2020. Overall Survival (OS), an interval between the date of biopsy and the last follow-up or the date of death, was also evaluated. The chemotherapeutic effect in the surgically resected samples was evaluated microscopically. Grade 0: Little change, grade 1a: Mild effect, grade 1b: Moderate effect, grade 2a: Marked effect but with viable cancer cells, grade 2b: Significant effect with only a few viable cancer cells, grade 3: No viable cancer cells seen (pCR: Pathological Complete Remission: Therapeutic effect grade 3). Nineteen (56%) of 34 TNBC lesions showed pCR. The TNBC lesions (n=34) were divided into two groups: 22 lesions showing marked response to NAC, grades 2b or 3, were categorized in the good responder, whereas 12 lesions with less response, grades 2a 1b 1a or 0, were categorized in the poor responder. The judgment was authorized by the General Rules for Clinical and Pathological Recording of Breast Cancer, ver. 18, 2018 published by the Japanese Breast Cancer Society [56].

Adjuvant (post-operative) chemotherapy was administered in three cases of basal-like TNBC in the non-NAC group. In three stage II cases of eight basal-like TNBC in the poor responder, additional adjuvant chemotherapy with EC, nabPTX or CMF (cyclophosphamide/methotrexate/5-fluorouracil) was administered. No adjuvant chemotherapy was given for the good responder. Since 2017, adjuvant capecitabine administration became common after NAC against TNBC [57]. The present analysis was done using cases in 2011 through 2016.

Table 2 summarizes clinicopathological features for the good responder, poor responder and non-NAC group, as well as for basal-like, apocrine-type and other types of TNBC.

Histopathological Evaluation

The specimens of a total of 34 TNBC tissues prior to NAC obtained by both core needle biopsy and surgery after NAC were evaluated microscopically. For TNBC lesions without NAC (n=17), both needle biopsy and surgical specimens were examined. The tissues were fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections at 4 μm thickness were mounted onto 3-aminopropyltriethoxysilane-coated glass slides. Hematoxylin and Eosin (HE)-stained sections were evaluated for histopathological features. TILs were microscopically judged as immune cells (small lymphocytes and plasma cells) seen in or around invasive cancer nests, according to the international guideline [40]. TILs were judged positive when more than 50% stromal area in the cancer tissue was occupied by the immune cells. Lymphoid infiltrates only at the invasion front or around the intraductal cancer lesion were not regarded as TILs.

Immunohistochemical Evaluation

The amino acid polymer technique (Simple Stain Max-PO-MULTI, Nichirei Bioscience, Tokyo, Japan) was utilized for immunostaining of ER, PgR, AR, HER2, p53 oncoprotein, Ki-67, epidermal growth factor receptor (EGFR or HER1), cytokeratin 5/6 (CK5/6), CK14 and gross cystic disease fluid protein-15 (GCDFP15). For demonstrating FOXA1, biotin-free catalyzed signal amplification-II modification provided by Agilent Technologies (Santa Clara, CA, USA) was employed. The antibodies and the soaking solution for heat-induced epitope retrieval by pressure pan heating for 10 minutes were described in our previous reports [16,24].

The 10% criterion was used for the judgment of hormone receptors (ER, PgR and AR), HER2, EGFR, CK5/6, CK14 and GCDFP15. When immunohistochemical positivity of HER2 was judged as 2+, fluorescence *in situ* hybridization study for HER2 genome was performed. For FOXA1, diffuse nuclear staining was judged positive, but cytoplasmic reactivity was not evaluated (focal nuclear positivity of FOXA1 was not experienced). Regarding Ki-67 labeling, the mean (not hot-spot) percentage of Ki-67 nuclear positivity was evaluated in invasive lesions in a stepwise fashion, such as 1, 2, 3, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90% [16]. p53 overexpression was regarded as positive when more than one third of the nuclei of cancer cells were positive.

Microscopic Characterizations of Apocrine- and Basal-Like Phenotypes

Apocrine metaplasia consistently lacks the expression of ER and PgR but expresses AR and FOXA1 in the nuclei. Figure 2 illustrates apocrine metaplasia seen in intraductal papilloma. Apocrine-type breast cancer reflects the nature of apocrine metaplasia of non-cancerous mammary tissue.

Table 3 summarizes the results of immunostaining. ER and PgR were consistently negative in the TNBC lesions. HER2 expression was judged as negative or 1+, except for two 2+ lesions (one in the good responder and one in the poor responder). Fluorescence *in situ* hybridization study failed to identify HER2 gene amplification in these two lesions.

Table 2. Summary of clinicopathological features. Footnotes: Good-R: Good Responders, Poor-R: Poor Responders.

		Good-R (n=22)	Poor-R (n=12)	Non-NAC (n=17)	Basal-like (n=29)	Apocrine (n=16)	others (n=6)
Basal-like		18	8	3			
Apocrine-type		2	4	10			
Others		2	0	4			
Age	≤ 49 years	13	4	3	15	2	3
	≥ 50 years	9	8	14	14	14	3
Stage	I	11	5	14	13	13	4
	IIA	10	4	3	12	3	2
	IIB	1	3	0	4	0	0
Tumor size	≤ 9 mm	2	1	4	1	4	2
	10-19 mm	10	7	7	14	9	1
	≥ 20 mm	10	4	6	14	3	3
H. grade	G1	0	1	6	0	7	0
	G2	4	6	8	5	8	5
	G3	18	5	3	24	1	1
Ki-67	≤ 40%	3	8	14	6	14	5
	≥ 50%	19	4	3	23	2	1
TILs	+	9	0	3	9	1	2
	-	13	12	14	20	15	4
DFS	≤ 59 months	12	5	7	12	9	3
	≥ 60 months	10	7	10	17	7	3
Prognosis	No recurrence	20	9	15	27	12	5
	Rec/alive	2	2	0	1	2	1
	Cancer death	0	1	1	1	1	0
	Non-ca. death	0	0	1	0	1	0

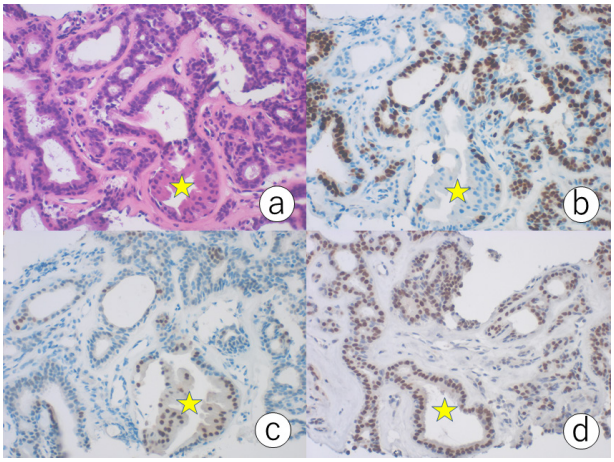

Figure 2. Apocrine metaplasia in intraductal papilloma (a: HE, b: ER, c: AR, d: FOXA1). Apocrine metaplastic cells (asterisks), expressing AR instead of ER, possess plump eosinophilic cytoplasm. Non-metaplastic duct epithelial cells are positive for ER but negative for AR. FOXA1 is expressed in both of the epithelial components.

Table 3. Summary of immunohistochemical features. Footnotes: Good-R: Good Responders, Poor-R: Poor Responders.

		Good-R (n=22)	Poor-R (n=12)	Non-NAC (n=17)	Basal-like (n=29)	Apocrine (n=16)	Others(n=6)
Ki-67	≥ 50%	19	4	3	23	2	1
	≤ 40%	3	8	14	6	14	5
AR	Positive	2	4	10	0	16	0
	Negative	20	8	7	29	0	6
FOXA1	Positive	3	5	11	1	16	2
	Negative	19	7	6	28	0	4
p53	Overexpressed	14	5	7	17	6	3
	Not expressed	8	7	10	12	10	3
EGFR	Expressed	19	7	14	25	11	4
	Not expressed	3	5	3	4	5	2
CK5/6	Expressed	20	10	10	27	8	5
	Not expressed	2	2	7	2	8	1
CK14	Expressed	11	4	7	17	1	4
	Not expressed	11	8	10	12	15	2
GCDFP15	Expressed	1	5	6	1	11	0
	Not expressed	21	7	11	28	5	6

In the NAC group, AR was expressed in the nuclei in six (18%) of 34 TNBC lesions, and eight (24%) showed diffuse nuclear expression of FOXA1. FOXA1 positivity without AR expression was seen in one basal-like lesion in the poor responder and one myoepithelial carcinoma in the good responder. In the apocrine-type lesion, both AR and FOXA1 were co-expressed, two (9%) in 22 good responders and four (33%) in 12 poor responders (p=0.15). The apocrine-type lesions showing high (50% or more) Ki-67 labeling were seen in two cases (one in the good responder and one in the poor responder). Exceptionally, one apocrine-type lesion with low (25%) Ki-67 index responded well to NAC (pCR).

In the non-NAC group, the ratio of the apocrine-type lesions was high: 10 (59%) of 17 lesions belonged to the apocrine-type (p<0.01). The age of the patients, including the both NAC and non-NAC group, was statistically older in the apocrine type than in the basal-like subtype (p<0.05). The age of the patients ranged from 38 to 87 years with the mean 62.8 and the median 62 for the apocrine-type TNBC (n=16), whereas it ranged from 34 to 71 years with the mean 53.7 and the median 49 for the basal-like subtype (n=29). The size of the tumor in the non-NAC group tended to be smaller than that in the NAC group, but no statistical significance was proven (p=0.20).

Special types phenotypically categorized as TNBC included myoepithelial carcinoma (n=3), medullary carcinoma (n=1), spindle cell carcinoma (n=1) and adenoid cystic carcinoma (n=1). One myoepithelial carcinoma and one medullary carcinoma belonged to the good responder. Two myoepithelial carcinomas, one adenoid cystic carcinoma and one spindle cell carcinoma were included in the non-NAC group. All the special types, except for medullary carcinoma, revealed low Ki-67 labeling indices.

The lesions other than the apocrine-type and the special types were regarded as the basal-like subtype. Three lesions (basal-like 1 and special type 2) expressed FOXA1 without AR positivity, including one spindle cell carcinoma in the non-NAC group. The basal-like subtype represented 18 (82%) of 22 good responders, 8 (67%) of 12 poor responders and three (18%) of 17 non-NAC group lesions (p<0.01). The basal-like lesions with high (50% or more) Ki-67 labeling represented 17 (94%) of 18 good responders, but three (38%) of eight poor responders (p<0.01).

It is noteworthy that TILs were associated in 10 (45%) of 22 good responders, including nine (50%) of 18 TNBC lesions of basal-like subtype and one medullary carcinoma, but no lesions were associated with TILs in the poor responder (p<0.01). TILs-positive TNBC consistently showed high Ki-67 labeling. In the non-NAC group, three lesions (one basal-like, one apocrine-type and one myoepithelial carcinoma) accompanied TILs. Among a total of 13 TILs-associated TNBC lesions, 10 (77%) belonged to TNBC of basal-like subtype. TILs were an excellent histological predictive marker for the responsiveness to NAC in TNBC, as was described previously [30-33].

Representative immunohistochemical features of TNBC of basal-like subtype and apocrine-type in the good responder are illustrated in Figures 3 and 4, respectively.

When the microscopic features were reviewed, apocrine-type TNBC commonly showed plump amphophilic cytoplasm, but TNBC of basal-like type often revealed indistinguishable histopathological appearance. Classical apocrine features with plump eosinophilic cytoplasm were seen in one poor responder and in six lesions in the non-NAC group with histological grade 1. Figure 5 demonstrates microscopic appearance of apocrine features in the poor responder group.

Expression of p53, EGFR, CK5/6, CK14 and GCDFP15

The results are summarized in Table 3. p53 was overexpressed in 17 (59%) of 29 basal-like lesions and six (38%) of 16 apocrine-type lesions (p=0.22). Expression of EGFR was relatively high in every group. CK5/6 positivity was significantly higher in the basal-like lesion (27/29=93%) than the apocrine-type lesion (8/16=50%) (p<0.05). CK14 expression was also significantly higher in the basal-like lesion (17/29=59%) than in the apocrine-type lesion (1/16=6%) (p<0.01). Expression of GCDFP15 was observed in one (3%) of 29 basal-like lesions and 11 (69%) of 16 apocrine-type lesions (p<0.01).

Regarding representative immunohistochemical features, refer to Figures 3-5.

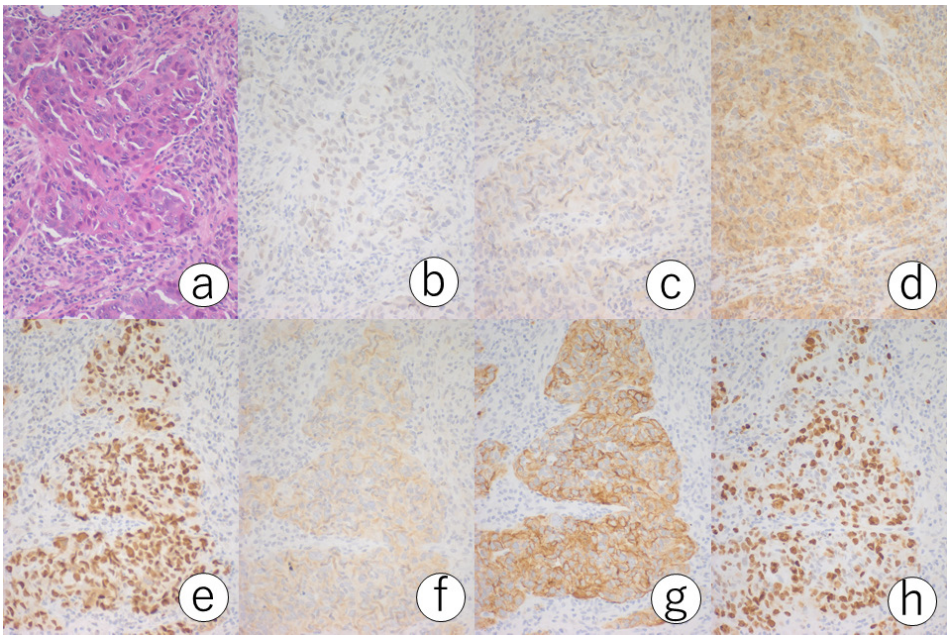

Figure 3. Immunohistochemical features of TNBC of basal-like subtype (a representative lesion in the good responder; a: HE, b: ER, c: AR, d: FOXA1, e: p53, f: EGFR, g: CK5/6, h: Ki-67). Highly atypical quadruple-negative cancer cells (histological grade 3) accompany TILs. ER, AR and FOXA1 are not expressed in the nuclei. Cytoplasmic positivity of FOXA1 is noted. p53, EGFR and CK5/6 are positive in the nuclei, on the plasma membrane and in the cytoplasm of the cancer cells, respectively. The EGFR reactivity is relatively weak in this case. Ki-67 labeling index is as high as 70%.

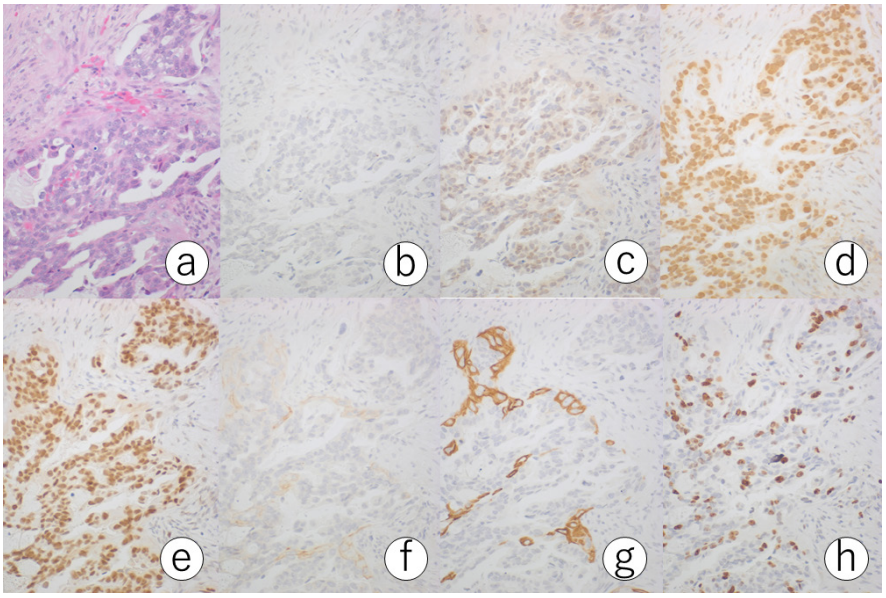

Figure 4. Immunohistochemical features of TNBC of apocrine-type (a representative lesion in the good responder; a: HE, b: ER, c: AR, d: FOXA1, e: p53, f: EGFR, g: CK5/6, h: Ki-67). Atypical ER-negative cancer cells forming a tubular structure (histological grade 2) express AR, FOXA1 and p53. EGFR shows focal reactivity. CK5/6 is immunoreactive in non-neoplastic myoepithelial cells, but the cancer cells are negative. Ki-67 labeling index is around 25%.

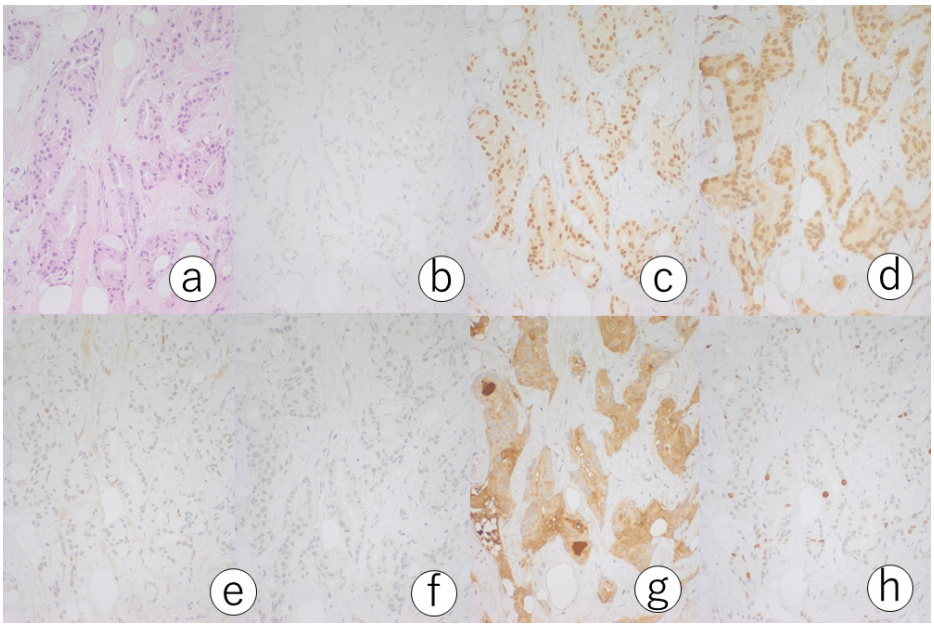

Figure 5. Immunohistochemical features of TNBC of apocrine-type (classical apocrine carcinoma in the poor responder; a: HE, b: ER, c: AR, d: FOXA1, e: p53, f: EGFR, g: GCDFP15, h: Ki-67). Mildly atypical cancer cells with eosinophilic cytoplasm and a tubular structure (histological grade 1) express AR and FOXA1, but ER, p53 and EGFR are negative. CK5/6 is not expressed (not shown). GCDFP is diffusely expressed. Ki-67 labeling index is low around 10%.

Immunohistochemical Definition of Apocrine-Type TNBC

We would like to emphasize the importance of immunohistochemical recognition of apocrine-type TNBC as triple-negative and AR-positive phenotypes. The incidence of apocrine-type breast cancer is much higher than expected under HE observation: 44 (13.5%) of 325 invasive ductal carcinomas, as reported previously [16]. It should be noted that histologically the apocrine-type breast cancer does not necessarily reveal typical apocrine appearance in HE preparations [16], and that apocrine carcinoma diagnosed under HE preparations alone may occasionally express ER (not apocrine immunohistochemically) [58,59]. Vranic, et al. proposed a strict definition of apocrine carcinoma of the breast as ER/PgR-negative and AR-positive invasive ductal carcinoma [60], in accordance with Tsutsumi's proposal [16]. HER2 overexpression rate in the apocrine-type invasive breast cancer was as high as 23/44 (52%), and the remaining half of apocrine-type breast cancer was thus categorized in TNBC. Genuine TNBC of basal-like subtype was quadruple-negative for ER, PgR, AR and HER2. It is noteworthy that the apocrine-type TNBC showed lower histological grading and lower Ki-67 labeling. Expression of p53 and basal cell markers (EGFR, CK5/6 and CK14) was common among both basal-like and apocrine-type TNBC [16]. Frequent expression of EGFR and CK5/6 in TNBC has been reported repeatedly [5,6,9]. In our present analysis, the apocrine-type lesions showed significantly low expression of CK5/6 and CK14 and significantly high GCDFP15 positivity when compared with basal-like lesions.

Herein, we accepted a strict definition of apocrine-type TNBC as TNBC immunoreactive for both AR and FOXA1 in the nuclei [24-26]. As already mentioned in the Introduction, FOXA1 is an intranuclear transcription factor co-localizing with ER or AR in the normal and neoplastic cells of the breast

and prostate [28,29]. In fact, hormone receptor-positive cancer cells of the breast and prostate commonly express FOXA1 in the nuclei [30-32]. TNBC of basal-like subtype is featured by the lack of nuclear expression of FOXA1, whereas the cytoplasmic positivity is common. In the present analysis, one basal-like lesion in the poor responder and two special type lesions (myoepithelial carcinoma in the good responder and spindle cell carcinoma in the non-NAC group) exceptionally expressed FOXA1 in the nuclei but without hormone receptor positivity.

Gene expression profiling studies have subclassified TNBCs into several intrinsic subtypes [3-7]. The importance of the appropriate recognition of intrinsic subtypes in TNBC has repeatedly been emphasized [61,62]. HER2-negative molecular apocrine subtype or Luminal Androgen Receptor (LAR) subtype was proven to be a distinct molecular subtype of TNBC [33-38]. In the current analysis, AR was expressed in six (18%) of 34 TNBC lesions in the NAC group, and all of them showed distinct nuclear expression of FOXA1, categorized as the apocrine-type. By the molecular analysis, Lehmann, et al. found the LAR subtype in 12% or 16% of TNBC [36,63]. Liu, et al. reported the LAR subtype in 29 (18%) of 165 TNBC lesions [38]. It has been shown that molecular apocrine breast cancer was ER-negative and expressed AR and FOXA1 [25,64], whereas 10% of molecular apocrine breast cancer lacked FOXA1 expression [64]. Co-expression of AR and FOXA1 in apocrine-type TNBC has also been described by independent research groups [21,26,65]. Nakashoji, et al. reported that chemosensitive TNBC tended to show low expression rates of AR and FOXA1 [51]. It is reasonable to regard that apocrine-type TNBC immunoreactive for both AR and FOXA1 represents the HER2-negative molecular apocrine or LAR subtype defined by the molecular study.

AR Expression in TNBC

AR is expressed in 53-90% of breast cancers [16,66-69]. Hickey, et al. suggested that AR signaling exerts an anti-estrogenic/growth inhibitory influence in ER-positive breast cancer [70]. By a meta-analysis, AR expression in breast cancer predicted favorable DFS, and better OS was noted in ER-positive cases [71]. The complexities of AR signaling in breast cancer were reviewed by McNamara, et al. [72]. AR expression in TNBC has also been studied extensively, and the AR positivity rate in TNBC ranged from 6.6 to 75% [17-23]. Rahim, et al. meta-analyzed 7,693 cases in 19 researches, and reported AR was expressed in 31.8% of ER-negative breast cancer [68]. In the present analysis, the apocrine-type represented 31% (16/51) of the TNBC lesions analyzed. The rate of BRCA mutations in TNBC of basal-like subtype was much higher than that in AR-positive TNBC [73].

Low aggressiveness of AR-positive TNBC has repeatedly been reported [17-23]. However, some researchers described controversial findings: Lehmann-Che, et al. suggested aggressiveness of molecular apocrine breast cancer [64], and Choi, et al. described decreased survival in cases with AR-positive TNBC [74]. Guiu, et al. suggested a worse outcome of AR-positive/FOXA1-positive TNBC compared to other TNBC, and a higher risk of the late recurrence [65]. Liu, et al. found no significant correlation between AR expression and NAC effect in TNBC, and the prognosis was poor in AR-positive TNBC in stage III [75]. In contrast, the recent molecular analysis has indicated low aggressiveness of the LAR subtype [25,36-38]. HER2-negative molecular apocrine or LAR-type TNBC showed a favorable prognosis despite chemoresistance [50,62,63]. In the current analysis using stages I and II cases, three (19%) of 16 apocrine-type lesions recurred and one patient died, whereas two (7%) of 29 basal-like lesions in the poor responder recurred and one died (p=0.31). This may represent both an excellent response to NAC in the basal-like lesion and low chemosensitivity of the apocrine-type lesion.

Table 4. Chemotherapeutic effects against TNBC (n=34) in relation to Ki-67 labeling index.

			0/G1a	G1b	G2a	G2b	G3
Ki-67	<50%	(n=11)	2	4	2	0	3
Ki-67	≥ 50%	(n=23)	3	0	1	3	16

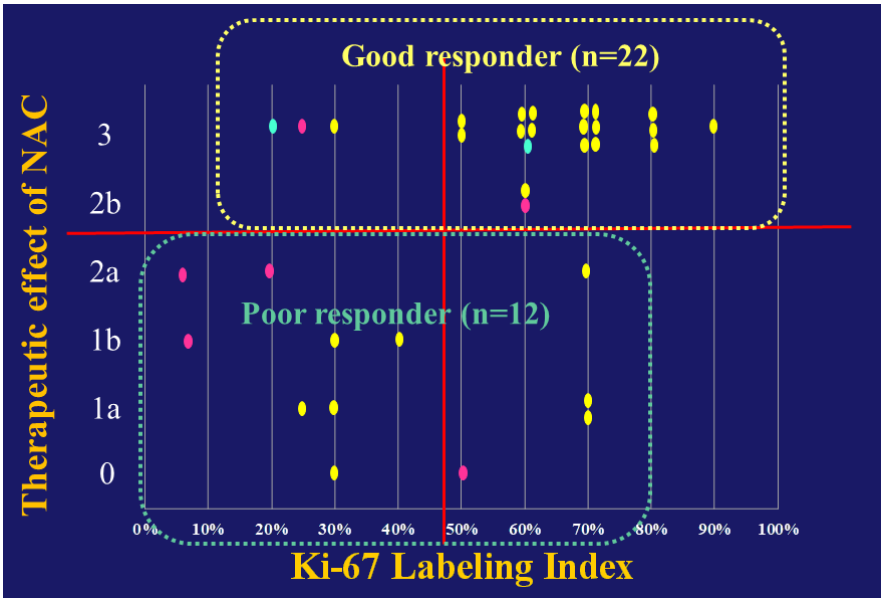

Figure 6. Schematic illustration of the relationship between the chemotherapeutic effect of NAC and Ki-67 labeling indices in TNBC (n=34), including 22 good responders and 12 poor responders. TNBCs with high Ki-67 labeling (50% or more) showed a good response to NAC, whereas those of low Ki-67 labeling (<50%) were frequently included in the poor responder. Statistical significance (p<0.01) was proven. **Note:** (▯) Basal-like type; (▯) Apocrine type; (▯) Other type

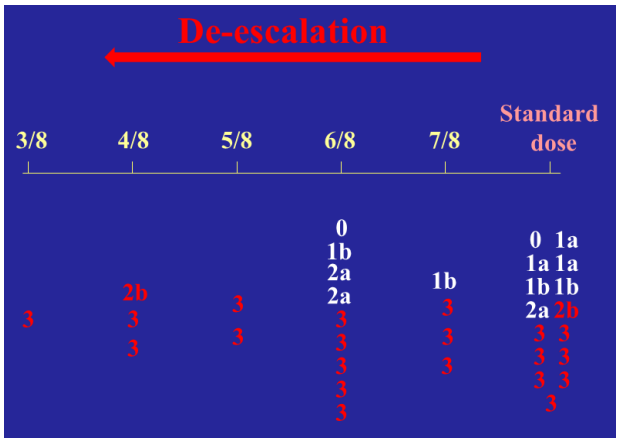

Figure 7. The number of cycles of NAC and therapeutic effect against TNBC in stages I&II (n=34). Standard dose means four cycles for A (anthracycline-based regimen) and four cycles for T (taxane-based regimen), namely 4+4=8 (standardized NAC). The fractions 3/8 through 7/8 indicate total cycles of A plus T (numerator) per standard dose (denominator). The total cycle numbers (numerators) smaller than 8 represent de-escalation (period-shortening) of the NAC. Good responders (G2b and G3=pCR) are shown in red. White figures (G1, G1a, G1b and 2a) represent poor responders. In most cases, the cycle of T was shortened. In five cases in the good responder, G3 or G2b was reached just after three or four cycles of anthracycline administration. The de-escalation of NAC was achieved in 14 (64%) of 22 cases of the good responder.

Ki-67 Labeling Index in Good and Poor Responders

Regarding clinicopathological features of the patients in the good responders, poor responders and non-NAC group, see Table 2. Total mastectomy was performed in four good responders with BRCA mutations (basal-like 2, apocrine-type 1 and myoepithelial carcinoma 1). The BRCAness was not recognized in the other lesions. All four stage IIB patients in the NAC group suffered basal-like TNBC, and three of them were categorized in the poor responder.

TNBC of basal-like subtype in the good responder (n=18) showed no recurrence after NAC, whereas apocrine-type TNBC revealed the chemoresistance. Three of four stage IIB cases of basal-like TNBC were categorized in the poor responder, indicating the significance of staging of the lesion.

The apocrine-type lesions comprised two (9%) of 22 good responders, and four (33%) of 12 poor responders (p=0.15). The apocrine-type lesions showing high Ki-67 labeling were seen in two cases (one in the good responder and one in the poor responder). Exceptionally, one apocrine-type with low Ki-67 index well responded to NAC. More than half cases in the non-NAC group (10/17=59%) belonged to the apocrine-type TNBC with low (<50%) Ki-67 labeling. Fourteen (88%) of 16 apocrine-type TNBC lesions evaluated showed low Ki-67 labeling.

In contrast, 23 (79%) of 29 basal-like TNBC lesions accompanied high (≥ 50%) Ki-67 labeling (p<0.01). It is noteworthy that five (63%) of eight poor responders with basal-like TNBC showed low Ki-67 labeling and no recurrence was recorded for 80 median months (mean 68.2, range: 39-93). Adjuvant chemotherapy was performed in three of these five cases. Two of three basal-like TNBC cases with high Ki-67 labeling in the poor responder recurred locally, and one died.

We should emphasize that Ki-67 labeling index well correlated with the responsiveness to NAC. In fact, TNBC with Ki-67 labeling 50% or more significantly responded well to NAC and was often categorized in the good responder, when compared with TNBC with low (<50%) Ki-67 labeling, as summarized in Table 4. In fact, 19 (83%) of 23 lesions of TNBC with high (≥ 50%) Ki-67 labeling showed G2b or G3 effect of NAC, whereas only 3 (27%) of 11 TNBC lesions with low (<50%) Ki-67 labeling revealed G3 effect (p<0.01). Figure 6 displays schematic relationship between the chemotherapeutic effect of NAC and Ki-67 labeling indices of TNBC (n=34). We propose that 50% labeling of Ki-67 should be regarded as the threshold level for judging the effectiveness of NAC. The 50% threshold level is much higher than Ki-67 labeling at 14-30% as the judging criteria for adjuvant chemotherapy against breast cancer of luminal types [76-80]. Miyashita, et al. proposed a Ki-67 cut-off value at 35% for TNBC for prognostic scoring [81]. Gass, et al. adopted 36% as the threshold value [82], and Santonja, et al. proposed the cut-off value at 50% in accordance with ours [62].

Evaluation of the Prognosis of Patients

The good responder to NAC contained lesions with therapeutic effects G2b (three lesions) and G3 (19 lesions). All the cases of the basal-like subtype (n=18) were alive without recurrence, and two cases revealed local recurrence (apocrine-type with G2b effect and myoepithelial carcinoma with G3 effect). The clinical outcome of cases with therapeutic effect G2b (a small volume of viable invasive cancer cells remaining after NAC) was comparable with cases with G3 effect. Among three cases with G2b effect, one apocrine-type lesion showed local recurrence without adjuvant chemotherapy. The data were as comparable as 19 cases with G3 effect: Local recurrence was recorded in one myoepithelial carcinoma (p=0.26).

A total of 26 patients with basal-like TNBC in the NAC group, 18 good responders and eight poor responders, were followed up for the mean period of 71.4 months (median 70.5) ranging from 30 to 113. Two basal-like lesions in the poor responder with high Ki-67 labeling, adjuvant chemotherapy given, showed multifocal local recurrence at the 12th month and at the 38th month, and the former patient died at the 30th month. As a result, all but one (96%) patients with basal-like TNBC were alive. Among 20 basal-like lesions with high (≥ 50%) Ki-67 labeling, 17 (85%) were categorized in the good responder and three (15%) in the poor responder. Among six basal-like lesions with low Ki-67 labeling, one responded to NAC (G2b), whereas the remaining five belonged to the poor responder. It is noteworthy that no recurrence was recorded after surgery in these six cases: The follow-up period ranged from 39 to 101 months with the mean 73.7 and the median 80.5.

All six patients with apocrine-type lesions, two in the good responder and four in the poor responder, were alive after the mean follow-up period of 73.5 months (the median 72.5) ranging from 34 to 108. Two cases (one in the good responder and one in the poor responder) showed local recurrence at the 12th month and at the 44th month, respectively. In the non-NAC group, three of 17 cases revealed the basal-like lesions with high Ki-67 labeling (one with TILs), and no recurrence was recorded for 88, 99 and 119 months after adjuvant chemotherapy. Ten (59%) of 17 lesions in the non-NAC group belonged to the apocrine-type with low Ki-67 labeling, and four belonged to the special types. One apocrine-type lesion in stage I recurred at the 20th month and died after 78 months. In this case, the recurrent tumor in the axillary node showed subtype conversion to ER/PgR-negative, AR-positive

but with overexpression of HER2. Cancer-unrelated death was recorded at the 19th month in another apocrine-type case aged 87 years.

In the past five years, we dared to avoid NAC in cases with apocrine- and special-type TNBC in stages I and II with low (<50%) Ki-67 labeling. These included four apocrine-type lesions, one spindle cell carcinoma and one myoepithelial carcinoma in the non-NAC group. The tumor size ranged from 6 to 40 mm (mean 21.5 mm, median 20.5). In these six cases, no recurrence has been experienced, although the follow-up period still remains short, ranging from 32 to 60 months (mean: 43.8, median 38.5).

The remaining 10 cases in the non-NAC group, including basal-like TNBC with high Ki-67 labeling and effective adjuvant chemotherapy (n=3), and TNBC of apocrine- (n=5) or special type (n=2) with low Ki-67 labeling, showed longer follow-up periods (mean 99.5, median 103, range: 67-129). Fifteen (94%) of 16 cases in the non-NAC group, excluding one case with cancer-unrelated death in the aged, were alive without recurrence.

The prognosis of the patients with the good responder, poor responder and non-NAC group was evaluated by Kaplan-Meier's method. The prognosis was compared by the logrank test. Bonferroni's correction was introduced when comparing three groups. No significant difference was observed for OS (p=0.308), as well as for DFS (p=0.321).

Our study included three myoepithelial carcinomas, one medullary carcinoma, one spindle cell carcinoma and one adenoid cystic carcinoma expressing TNBC features. In addition to medullary carcinoma, one myoepithelial carcinoma responded well to NAC. No NAC was administered to the remaining cases. The importance of appropriate histopathological recognition of such special types with TNBC phenotype should be emphasized [12-14, 51-54].

Period-Shortening (de-escalation) of NAC in Basal-Like TNBC with High Ki-67 Labeling Index

When contrast-enhanced MRI indicates cCR, the period of NAC can be shortened. We introduced MRI evaluation in an ad libitum fashion when the lesion was shrunken to 5 mm or less by ultrasound screening. In the course of NAC, cCR was effectively judged by contrast-enhanced MRI [83]. Among 22 good responders, period shortening (de-escalation) of NAC was achieved as an unintentional result in 14 (64%) cases, as shown in Figure 7. In three cases in the good responder, pCR (therapeutic effect G3) was reached just after three or four cycles of anthracycline administration. One case showed G2b just after four anthracycline cycles. It is noteworthy that basal-like TNBC with high (≥ 50%) Ki-67 labeling was susceptible to anthracyclines. Two cases (apocrine-type TNBC and myoepithelial carcinoma) with BRCAness showed local recurrence after the de-escalated NAC and total mastectomy, but after local excision of the small-sized recurred tumor and radiotherapy, the patients remained disease-free for 22 and 21 months after recurrence without additional chemotherapy.

The de-escalation must be a significant advantage of NAC: In case of adjuvant chemotherapy, it is hard to de-escalate the period, because of the paucity of appropriate barometers. The idea of "relative dose intensity" indicates that the maintenance of the total amount of drug given per cycle ("dose intensity") at more than 85% of the standardized therapy is crucial to controlling cancers [84,85]. In our protocol, the dose intensity itself was maintained to achieve shortening of the dosing period.

Recent paradigm shift in breast cancer biology indicates changes of breast cancer management from radical mastectomy to precision (personalized) medicine. Both Halstedian and Fisherian hypotheses recommend certain fixed treatment protocol to all patients with breast cancer. Along with "the spectrum theory" as reviewed by Özmen [86], we should separate low-risk patients from high-risk patients to avoid overtreatment for low-risk patients. It is likely that certain percentage of low-risk patients accompanying good prognostic factors do not require systemic and/or radiation therapy. The same hypothesis may be appliable to patients with TNBC accompanying higher or lower risks, as we suggested in the present analysis.

De-escalation of NAC should be imperious for the patients to relieve cytotoxic drug-induced serious adverse reactions, such as cardiac toxicity by anthracycline and peripheral neuropathy and dysgeusia by taxane. The patient-friendly chemotherapy will also contribute to health economics by saving medical expenses.

Therapeutic Strategy for Apocrine-Type TNBC

The effectiveness of anti-androgen therapy against apocrine-type breast cancer has been described repeatedly [18,19,50,61,63,66,68,87,88]. We cordially expect introduction of anti-androgen therapy against apocrine-type TNBC. Arce-Salinas, et al. reported the usefulness of AR antagonist bicalutamide for the treatment of metastatic AR-positive TNBC [89]. Effectiveness of an AR blocker, enzalutamide, against AR-positive TNBC has been reported [90]. Hilborn, et al. described beneficial tamoxifen response in ER-negative and AR-positive breast cancer [91]. Hormonal therapy strategy against apocrine-type TNBC should thus be reappraised.

Recently, Bareche, et al. identified frequent (75%) somatic mutations in the PI3K (phosphatidylinositol 3-kinase)- AKT (v-Akt murine thymoma viral oncogene) signaling pathway in the LAR subtype of TNBC, and proposed possible use of a PI3K/AKT inhibitor as the molecular target therapy for AR-positive TNBC [92]. Appropriate recognition of this unique subtype of TNBC distinguished by adding immunostaining for AR and FOXA1 in the routine panel must be emphasized again.

Conclusion

We propose herein two major hypothetical schemes. Immunohistochemical recognition of apocrine-type TNBC with low Ki-67 labeling is critically important for avoiding ineffective and unnecessary NAC. Basal-like TNBC with high Ki-67 labeling is highly susceptible to NAC to achieve period-shortening (de-escalation) of NAC.

Limitations and recommendations for future studies

The present analysis belonged to a small-scale retrospective analysis, performed in a local clinic in Yokohama, Japan. Large-scale prospective clinical trials are needed to confirm our hypothetical proposals.

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Funding and Conflict of Interest

The review was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Issue

The research conformed to the provision of the Declarations of Helsinki in 1995. The study protocol was approved in 2016 by the Ethics Committee for Medical Research of Fujita Health University, Toyoake, Japan (#HM16-028), and Yokohama Breast and Gastrointestinal Clinic, Yokohama, Japan. Written informed consent was obtained from each patient, after the approval of our study protocol. All the histopathological and immunostaining data in the signed histopathological diagnostic report by a pathologist (Y.T.) were explained for the patient when the treatment plan was presented by attending physicians (K.K. and K.S.). Since our treatment plan was not necessarily in accordance with the internationally standardized therapeutic discipline, all patients were also verbally informed of the investigational nature of the present study, when they visited the outpatient clinic. The verbal informed consent was recorded in the clinical chart.

References

1. Ferlay, Jacques, Isabelle Soerjomataram, Rajesh Dikshit and Sultan Eser, et al. "Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Pattern in GLOBOCAN 2012." *Int J Cancer* 136 (2015): E359-E386.
2. Center for Cancer Control Information Services, National Cancer Center. "*Cancer Statistics in Japan*." (2021).
3. Perou, Charles M, Therese Sørlie, Michael B. Eisen and Matt van de Rijn, et al. "Molecular Portraits of Human Breast Tumours." *Nature* 406(2000): 747-752.
4. Sorlie, Therese, Robert Tibshirani, Joel Parker and Trevor Hastie, et al. "Repeated Observation of Breast Tumor Subtypes in Independent Gene Expression Data Sets." *Proc Natl Acad Sci USA* 100 (2003): 8418-8423.
5. Nielsen, Torsten O, Forrest D Hsu, Kristin Jensen and Maggie Cheang, et al. "Immunohistochemical and Clinical Characterization of the Basal-Like Subtype of Invasive Breast Carcinoma." *Clin Cancer Res* 10 (2004): 5367-5374.
6. Livasy, Chad A, Gamze Karaca, Rita Nanda and Maria S Tretiakova, et al. "Phenotypic Evaluation of the Basal-Like Subtype of Invasive Breast Carcinoma." *Mod Pathol* 19 (2006): 264-271.
7. Cuzick, Jack, Mitch Dowsett, Silvia Pineda and Christopher Wale, et al. "Prognostic Value of a Combined Estrogen Receptor, Progesterone Receptor, Ki-67, and Human Epidermal Growth Factor Receptor 2 Immunohistochemical Score and Comparison with the Genomic Health Recurrence Score in Early Breast Cancer." *J Clin Oncol* 29 (2011): 4273-4278.
8. Inwald, Elisabeth Christine, M Koller, M Klinkhammer-Schalke and F Zeman, et al. "4-IHC Classification of Breast Cancer Subtype in a Large Cohort of A Clinical Cancer Registry: Use In Clinical Routine For Therapeutic Decisions and its Effect On Survival." *Breast Cancer Res Treat* 153 (2015): 647-658.
9. Tang, Ping and Gary M. Tse. "Immunohistochemical Surrogates for Molecular Classification of Breast Carcinoma. A 2015 Update." *Arch Pathol Lab Med* 140 (2016): 806-814.
10. Kassam, Farrah, Katherine Enright, Rebecca Dent and George Dranitsaris, et al. "Survival Outcomes for Patients with Metastatic Triple Negative Breast Cancer: Implications for Clinical Practice and Trial Design." *Clin Breast Cancer* 9 (2009): 29-33.
11. Gelmon K, R Dent, JR Mackey and K Laing, et al. "Targeting Triple-Negative Breast Cancer: Optimizing Therapeutic Outcomes." *Ann Oncol* 23 (2012): 2223-2234.
12. Liedtke, Cornelia, Chafika Mazouni, Kenneth R Hess and Attila Tordai, et al. "Response to Neoadjuvant Therapy and Long-Term Survival in Patients with Triple-Negative Breast Cancer." *J Clin Oncol* 26 (2008): 1275-1281.
13. Cortazar, Patricia, Lijun Zhang, Michael Untch and Keyur Mehta, et al. "Pathological Complete Response and Long-Term Clinical Benefit in Breast Cancer: The CTNeoBC Pooled Analysis." *Lancet* 384 (2014): 164-172.
14. Chen, Victor E, Erin F Gillespie, Kaveh Zakeri and James D Murphy, et al. "Pathologic Response after Neoadjuvant Chemotherapy Predicts Locoregional Control in Patients with Triple Negative Breast Cancer." *Adv Radiat Oncol* 2 (2017): 105-109.
15. Kubouchi, Koichi, Masaharu Kawaguchi, Kyosuke Shimada and Emiko Hasegawa, et al. "Neoadjuvant Chemotherapy against Triple-Negative Breast Cancer: Anticipation of the Therapeutic Effect and Selection of Therapy." *The 24th Meeting of the Japanese Breast Cancer Society, Tokyo,* 2016 (oral presentation).
16. Tsutsumi, Yutaka. "Apocrine Carcinoma as Triple-Negative Breast Cancer: Novel Definition of Apocrine-Type Carcinoma as Estrogen/Progesterone Receptor-Negative and Androgen Receptor-Positive Invasive Ductal Carcinoma." *Jpn J Clin Oncol* 42 (2012): 375-386.
17. Gucalp, Ayca and Tiffany A Traina. "Triple-Negative Breast Cancer: Role of the Androgen Receptor." *Cancer J* 16 (2010): 62-65.
18. McNamara, KM, Yoda T, Takagi K and Miki Y. "Androgen Receptor in Triple Negative Breast Cancer." *J Steroid Biochem Mol Biol* 133 (2013): 66-76.
19. Gasparini, Pierluigi, Matteo Fassan, Luciano Cascione and Gulnur Guler, et al. "Androgen Receptor Status is a Prognostic Marker in Non-Basal Triple Negative Breast Cancers and Determines Novel Therapeutic Options." *PLoS One* 9 (2014): e88525.
20. McGhan, Lee J, Ann E McCullough, Cheryl A Protheroe and Amylou C Dueck, et al. "Androgen Receptor-Positive Triple Negative Breast Cancer: A Unique Breast Cancer Subtype." *Ann Surg Oncol* 21 (2014): 361-367.
21. McNamara, Keely M, Tomomi Yoda, Alif Meem Nurani and Yukiko Shibahara, et al. "Androgenic Pathways in the Progression of Triple-Negative Breast Carcinoma: A Comparison between Aggressive and Non-Aggressive Subtypes." *Breast Cancer Res Treat* 145 (2014): 281-293.
22. Thike, Aye Aye, Luke Yong-Zheng Chong, Poh Yian Cheok and Hui Hua Li, et al. "Loss of Androgen Receptor Expression Predicts Early Recurrence in Triple-Negative and Basal-Like Breast Cancer." *Mod Pathol* 27 (2014): 352-360.
23. Rampurwala, Murtuza, Kari B Wisinski and Ruth O'Regan. "Role of the Androgen Receptor in Triple-Negative Breast Cancer." *Clin Adv Hematol Oncol* 14 (2016): 186-193.
24. Kubouchi, Koichi, Kyosuke Shimada, Takamichi Yokoe and Yutaka Tsutusmi. "Avoidance and Period-Shortening of Neoadjuvant Chemotherapy against Triple-Negative Breast Cancer in Stages I and II: Importance of Ki-67 Labeling Index and the Recognition of Apocrine-Type Lesions." *Technol Cancer Res Treat* 19 (2020): 1-16.
25. Robinson, Jessica LL, Stewart Macarthur, Caryn S Ross-Innes and Wayne D Tilley, et al. "Androgen Receptor Driven Transcription in Molecular Apocrine Breast Cancer is Mediated by FoxA1." EMBO J 30 (2011): 3019-3027.
26. Sasahara, Manami, Akira Matsui, Yoshiko Ichimura and Yuuko Hirakata, et al. "Overexpression of Androgen Receptor and Forkhead-box A1 Protein in Apocrine Breast Carcinoma." *Anticancer Res* 34 (2014): 1261-1267.
27. Lupien, Mathieu, Jérôme Eeckhoute, Clifford A. Meyer and Qianben Wang, et al. "FoxA1 Translates Epigenetic Signatures into Enhancer-Driven Linegane: Specific Transcription." *Cell* 132 (2008): 958-970.
28. Carroll, Jason S and Myles Brown. "Estrogen Receptor Target Gene: An Evolving Concept." *Mol Endocrinol* 20 (2006): 1707-1714.
29. Bernardo, Gina M and Ruth A Keri. "FOXA1: A Transcription Factor with Parallel Functions in Development and Cancer." *Biosci Rep* 32 (2012): 113-130.
30. Badve, Sunil, Dmitry Turbin, Mangesh A. Thorat and Akira Morimiya, et al. "FOXA1 Expression in Breast Cancer--Correlation with Luminal Subtype A and Survival." *Clin Cancer Res* 13 (2007): 4415-4421.
31. Albergaria, André, Joana Paredes, Bárbara Sousa and Fernanda Milanezi, et al. "Expression of FOXA1 and GATA-3 in Breast Cancer: The Prognostic Significance in Hormone Receptor-Negative Tumours." *Breast Cancer Res* 11 (2009): R40.
32. Robinson, Jessica LL and Jason S Carroll. "FoxA1 is a Key Mediator of Hormonal Response in Breast and Prostate cancer." *Front Endocrinol* (Lausanne) 3 (2012): 68.
33. Farmer, Pierre, Herve Bonnefoi, Veronique Becette and Michele Tubiana-Hulin, et al. "Identification of Molecular Apocrine Breast Tumours by Microarray Analysis." *Oncogene* 24 (2005): 4660-4671.
34. Doane, AS, Danso M, Lal P and Donaton M, et al. "An Estrogen Receptor-Negative Breast Cancer Subset Characterized by a Hormonally Regulated Transcriptional Program and Response to Androgen." *Oncogene* 25 (2006): 3994-4008.

35. Guedj, M, Marisa L, Reynies A de and Orsetti B, et al. "A Refined Molecular Taxonomy of Breast Cancer." *Oncogene* 31 (2011): 1196-1206.

36. Lehmann, Brian D, Bojana Jovanović, Xi Chen and Monica V. Estrada, et al. "Refinement of Triple-Negative Breast Cancer Subtypes: Implications for Neoadjuvant Chemotherapy Selection." *PLoS One* 11 (2016): e0157368.

37. Burstein, Matthew D, Anna Tsimelzon, Graham M Poage and Kyle R Covington, et al. "Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer." *Clin Cancer Res* 21 (2015): 1688-1698.

38. Liu, Yi-Rong, Yi-Zhou Jiang, Xiao-En Xu and Ke-Da Yu, et al. "Comprehensive Transcriptome Analysis Identifies Novel Molecular Subtypes and Subtype-Specific RNAs of Triple-Negative Breast Cancer." *Breast Cancer Res* 18 (2016): 33.

39. Ono, M, Tsuda H, Shimizu C and Yamamoto S, et al. "Evaluation of Tumor-Infiltrating Lymphocytes (TIL) and Tumor Cell Apoptosis as Predictive Markers for Response To Neoadjuvant Chemotherapy In Triple-Negative Breast Cancer." *J Clin Oncol* 27 (2009): 559-559.

40. Salgado, R, Denkert C, Demaria S and Sirtaine N, et al. "The evaluation of Tumor-Infiltrating Lymphocytes (TILs) in Breast Cancer: Recommendations by an International TILs Working Group 2014." *Ann Oncol* 26 (2015): 259-271.

41. Denkert, Carsten, Gunter von Minckwitz, Silvia Darb-Esfahani and Bianca Lederer, et al. "Tumour-Infiltrating Lymphocytes and prognosis in Different Subtypes of Breast Cancer: A Pooled Analysis of 3771 Patients Treated with Neoadjuvant Therapy." *Lancet Oncol* 19 (2018): 40-50.

42. Asano, Yuka, Shinichiro Kashiwagi, Wataru Goto and Koji Takada, et al. "Prediction of Treatment Response To Neoadjuvant Chemotherapy in Breast Cancer by Subtype Using Tumor-Infiltrating Lymphocytes." *Anticancer Res* 38 (2018): 2311-2321.

43. Leon-Ferre, Roberto Antonio, Mei-Yin Polley, Heshan Liu and Victoria Cafourek, et al. "Tumor-Infiltrating Lymphocytes in Androgen Receptor-Positive Triple-Negative Breast Cancer." *J Clin Oncol* 36 (2018): 5.

44. Fisher, B, Brown A, Mamounas E and Wieand S, et al. "Effect of Preoperative Chemotherapy on Local-Regional Disease in Women with Operable Breast Cancer: Findings from National Surgical Adjuvant Breast and Bowel Project B-18." *J Clin Oncol* 15 (1997): 2483-2493.

45. Rastogi, Priya, Stewart J Anderson, Harry D Bear and Charles E Geyer, et al. "Preoperative Chemotherapy: Updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27." *J Clin Oncol* 26 (2008): 778-785.

46. Bagegni, Nusayba A, Yu Tao and Foluso O. Ademuyiwa. "Clinical Outcomes with Neoadjuvant versus Adjuvant Chemotherapy For Triple Negative Breast Cancer: A Report From The National Cancer Database." *PLoS One* 14 (2019): e0222358.

47. Caln H, Macpherson IR, Beresford M, and Pinder SE, et al. "Neoadjuvant Therapy in Early Breast Cancer: Treatment Considerations and Common Debates in Practice." *Clin Oncol* 29 (2017): 642-652.

48. Loibl, Sibylle, Berit Maria Müller, Gunter von Minckwitz and Michael Schwabe, et al. "Androgen Receptor Expression in Primary Breast Cancer and its Predictive and Prognostic Value in Patients Treated with Neoadjuvant Chemotherapy." *Breast Cancer Res Treat* 130 (2011): 477-487.

49. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). "Guidance For Industry: Pathologic Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval." *Washington, DC: US Department of Health and Human Services* (2014).

50. Masuda, Hiroko, Keith A. Baggerly, Ying Wang and Ya Zhang, et al. "Differential Response to Neoadjuvant Chemotherapy among 7 Triple-Negative Breast Cancer Molecular Subtypes." Clin Cancer Res 19 (2013): 5533-5540.

51. Nakashoji, Ayako, Akira Matsui, Aiko Nagayama and Yuko Iwata, et al. "Clinical Precictors of Pathological Complete Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer." *Oncol Lett* 14 (2017): 4135-4141.

52. Nagao, T, Kinoshita T, Hojo T and Tsuda H, et al. "The Differences in the Histological Types of Breast Cancers and the Response to Neoadjuvant Chemotherapy: The Relationship between the Outcome and the Clinicopathological Characteristics." *Breast* 21 (2012): 289-295.

53. Tanabe, Yuko, Hitoshi Tsuda, Masayuki Yoshida and Mayu Yunokawa, et al. "Pathological Features of Triple-Negative Breast Cancers That Showed Progressive Disease during Neoadjuvant Chemotherapy." *Cancer Sci* 108(2017): 1520-1529.

54. Gradishar, William J, Benjamin O Anderson, Ron Balassanian and Sarah L Blair, et al. "NCCN Guidelines Insights: Breast Cancer, Version 1.2017. *J Natl Compr Canc Netw* 15 (2017): 433-451.

55. Shimoi, Tatsunori, Shigenori E Nagai, Tetsuhiro Yoshinami and Masato Takahashi, et al. "The Japanese Breast Cancer Society Clinical Practice Guideline for Systemic Treatment of Breast Cancer, 2018 Edition." *Breast Cancer* 27 (2020): 322-331.

56. The Japanese Breast Cancer Society. "The General Rules for Clinical and Pathological Recording of Breast Cancer (version 18)". *Kanehara Shuppan, Tokyo* (in Japanese) (2018).

57. Masuda, Norikazu, Soo-Jung Lee, Shoichiro Ohtani and Young-Hyuck Im, et al. "Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy." *N Engl J Med* 376 (2017): 2147-2159.

58. Gatalica, Zoran. "Immunohistochemical Analysis of Apocrine Breast Lesions: Consistent Over-Expression of Androgen Receptor Accompanied by the Loss of Estrogen and Progesterone Receptors in Apocrine Metaplasia and Apocrine Carcinoma *In Situ*." *Pathol Res Pract* 193 (1997): 753-758.

59. Sapp, Michelle, Abha Malik, and Wedad Hanna. "Hormone Receptor Profile of Apocrine Lesions of the Breast." Breast J 9 (2003): 335-336.

60. Vranic, Semir, Fernando Schmitt, Anna Sapino and Jose Luis Costa, et al. "Apocrine Carcinoma of the Breast: A Comprehensive Review." *Histol Histopathol* 28 (2013): 1393-1409.

61. Gerratana, L, Basile D, Buono G, and De Placido S, et al. "Androgen Receptor in Triple Negative Breast Cancer: A Potential Target for the Targetless Subtype." *Cancer Treat Rev* 68 (2018): 102-110.

62. Santonja, Angela, Alfonso Sánchez-Muñoz, Ana Lluch and Maria Rosario Chica-Parrado, et al. "Triple Negative Breast Cancer Subtypes and Pathologic Complete Response Rate to Neoadjuvant Chemotherapy." *Oncotarget* 9 (2018): 26406-26416.

63. Lehmann, Brian D, Joshua A Bauer, Xi Chen and Melinda E Sanders, et al. "Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies." *J Clin Invest* 121 (2011): 2750-2767.

64. Lehmann-Che, Jacqueline, Anne-Sophie Hamy, Raphaël Porcher and Marc Barritault, et al. "Molecular Apocrine Breast Cancers are Aggressive Estrogen Receptor Negative Tumors Overexpressing either HER2 or GCDFP15." *Breast Cancer Res* 15 (2013): R37.

65. Guiu, Séverine, Céline Charon-Barra, Déwi Vernerey and Pierre Fumoleau, et al. "Coexpression of Androgen Receptor and FOXA1 in Nonmetastatic Triple-Negative Breast Cancer: Ancillary Study from PACS08 trial." *Future Oncol* 11 (2015): 2283-2297.

66. Moinfar, Farid, Murat Okcu, Oleksiy Tsybrovskyy and Peter Regitnig, et al. "Androgen Receptors Frequently are Expressed in Breast Carcinomas: Potential Relevance to New Therapeutic Strategies." *Cancer* 98 (2003): 703-711.

67. Ogawa, Yoshinari, Eishu Hai, Kanako Matsumoto and Katsumi Ikeda, et al. "Androgen Receptor Expression in Breast Cancer: Relationship with Clinicopathological Factors and Biomarkers." *Int J Clin Oncol* 13 (2008): 431-435.

68. Rahim, Bilal and Ruth O'Regan. "Androgen Receptor Signaling in Breast Cancer." *Cancers (Basel)* 9 (2017): 21.

69. Vranic, Semir, Rebecca Feldman, and Zoran Gatalica. "Apocrine Carcinoma of the Breast: A Brief Update on the Molecular Features and Targetable Biomarkers." *Bosn J Basic Med Sci* 17 (2017): 9-11.

70. Hickey, TE, JL Robinson, JS Carroll and WD Tilley. "Minireview: The Androgen Receptor in Breast Tissues: Growth Inhibitor, Tumor Suppressor, Oncogene?" *Mol Endocrinol* 26 (2012): 1252-1267.

71. Qu, Qing, Yan Mao, Xiao-chun Fei and Kun-we Shen. "The Impact of Androgen Receptor Expression on Breast Cancer Survival: A Retrospective Study and Meta-Analysisi." *PLoS One* 8 (2013): e826.

72. McNamara, Keely M, Nicole L Moore, Theresa E Hickey and Hironobu Sasano, et al. "Complexities of Androgen Receptor Signaling in Breast Cancer." *Endocr Relat Cancer* 21 (2014): T161-T181.

73. Tung, Nadine M, Judy Ellen Garber, Vanda Torous and Michele R Hacker, et al. "Prevalence and Predictors of Androgen Receptor (AR) and Programmed Death-Ligand 1 (PD-L1) in BRCA1-Associated and Sporadic Triple-Negative Breast Cancer (TNBC)." *NPJ Breast Cancer* 2 (2016): 16002.

74. Choi, Jung Eun, Su Hwan Kang, Soo Jung Lee, and Young Kyung Bae. "Androgen Receptor Expression Predicts Decreased Survival in Early Stage Triple-Negative Breast Cancer." *Ann Surg Oncol* 22 (2015): 82-89.

75. Liu, Ya-Xuan, Ke-Jing Zhang and Li-Li Tang. "Clinical Significance of Androgen Receptor Expression in Triple-Negative Breast Cancer: An Immunohistochemical Study." *Oncol Lett* 15 (2018): 10008-10016.

76. Dowsett, Mitch, Torsten O Nielsen, Roger A'Hern and John Bartlett, et al. "Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group." *J Natl Cancer Inst* 103 (2011): 1656-1664.

77. Inic, Zorka, Milan Zegarac, Momcilo Inic and Ivan Markovic, et al. "Difference between Luminal A and Luminal B Subtypes According to Ki-67, Tumor Size, and Progesterone Receptor Negativity Providing Prognostic Information." *Clin Med Insights Oncol* 8 (2014): 107–111.

78. Denkert, Carsten, Jan Budczies, Gunter von Minckwitz and Stephan Wienert, et al. "Strategies for Developing Ki67 as a Useful Biomarker in Breast Cancer." *Breast* 24 (2015): S67-S72.

79. Bustreo, Sara, Simona Osella-Abate, Paola Cassoni and Michela Donadio, et al. "Optimal Ki67 cut-off for Luminal Breast Cancer Prognostic Evaluation: A Large Case Series Study with a Long-Term Follow-up." *Breast Cancer Res Treat* 157 (2016): 363-371.

80. Ács, Balázs, Veronika Zámbó, Laura Vízkeleti, and A Marcell Szász, et al. "Ki-67 as a Controversial Predictive and Prognostic Marker in Breast Cancer Patients Treated with Neoadjuvant Chemotherapy." *Diagn Pathol* 12 (2017): 20.

81. Miyashita, Minoru, Takanori Ishida, Kazuyuki Ishida and Kentaro Tamaki, et al. "Histopathological Subclassification of Triple Negative Breast Cancer Using Prognostic Scoring System: Five Variables as Candidates." *Virchows Archiv* 458 (2011): 65-72.

82. Gass, Paul, Michael P Lux, Claudia Rauh and Alexander Hein, et al. "Prediction of Pathological Complete Response and Prognosis in Patients with Neoadjuvant Treatment for Triple-Negative Breast Cancer." *BMC Cancer* 18 (2018): 1051.

83. Vaidya, Jayant S, Samuele Massarut, Hrisheekesh J Vaidya and Emma C Alexander, et al. "Rethinking Neoadjuvant Chemotherapy for Breast Cancer." *BMJ* 360 (2018): j5913.

84. Raza, S, Welch S and Younus J. "Relative Dose Intensity Delivered To Patients with Early Breast Cancer: Canadian Experience." *Curr Oncol* 16 (2009): 8-12.

85. Wildiers, Hans and Marcel Reiser. "Relative Dose Intensity of Chemotherapy and Its Impact on Outcomes in Patients with Early Breast Cancer or Aggressive Lymphoma." *Crit Rev Oncol Hematol* 77 (2011): 221-240.

86. Özmen, Vahit. "Paradigm Shift from Halstedian Radical Mastectomy To Personalized Medicine." *J Breast Health* 13 (2017): 50-53.

87. Ni, Min, Yiwen Chen, Elgene Lim and Hallie Wimberly, et al. "Targeting Androgen Receptor in Estrogen Receptor-Negative Breast Cancer." *Cancer Cell* 20 (2011): 119-131.

88. Kono, Miho, Takeo Fujii, Bora Lim and Meghan Sri Karuturi, et al. "Androgen Receptor Function and Androgen Receptor–Targeted Therapies in Breast Cancer: A Review." *JAMA Oncology* 3 (2017): 1266-1273.

89. Arce-Salinas, Claudia, Maria Carmen Riesco-Martinez, Wedad Hanna and Philippe Bedard Ellen Warner. "Complete Response of Metastatic Androgen Receptor–Positive Breast Cancer to Bicalutamide: Case Report and Review of the Literature." *J Clin Oncol* 34 (2016): e21-e24.

90. Traina, Tiffany A, Kathy Miller, Denise A Yardley and Janice Eakle, et al. "Enzalutamide for the Treatment of Androgen Receptor-Expressing Triple-Negative Breast Cancer." *J Clin Oncol* 36 (2018): 884-890.

91. Hilborn, Erik, Jelena Gacic, Tommy Fornander and Bo Nordenskjöld, et al. "Androgen Receptor Expression Predicts Beneficial Tamoxifen Response in Oestrogen Receptor-α-Negative Breast Cancer." *Br J Cancer* 114 (2016): 248-255.

92. Bareche, Y, Venet D, Ignatiadis M and Aftimos P, et al. "Unravelling Triple-Negative Breast Cancer Molecular Heterogeneity Using an Integrative Multiomic Analysis." *Ann Oncol* 29 (2018): 895-902.

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