

# Soluble IL-2R as a predictor of familial breast cancer

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## Research article

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# Abstract

**Background:** The incidence of breast cancer has been increasing annually, and breast cancer-related diseases, such as breast cancer in the young, ovarian cancer, prostate cancer, and pancreatic cancer, have clearly and steadily increased in number and have become common among the family members of patients with breast cancer. Accordingly, an increase in the incidence of familial breast cancer (FBC) is anticipated in the future. Interleukin (IL)-2 is one of the cytokines that activate cytotoxic T lymphocytes (CTLs), which are important for cancer immunity. To identify the markers of increased risk for FBC, soluble IL-2 receptor (sIL-2R) levels and immunologic factors were investigated in patients with FBC and non-familial breast cancer (NFBC).

**Methods:** Of the 106 untreated breast cancer patients who gave consent to participate in this study, 24 had FBC and 82 had NFBC. There were 11 healthy individuals included in this study. Serum and peripheral blood mononuclear cells were collected from all patients for measurement of sIL-2R, IL-10, Vascular endothelial growth factor (VEGF), IL-17, regulatory T cells (Tregs), Myeloid derived suppressor cells (MDSCs), neutrophil to lymphocyte ratio (NLR), white blood cell (WBC) and C-reactive protein (CRP) levels. Prognosis was assessed and compared according to sIL-2R levels (low vs. high). Tissue samples from postoperative patients with high sIL-2R levels were stained with programmed cell death ligand 1 (PD-L1) and Cluster of Differentiation (CD) 8.

**Results:** sIL-2R level was significantly higher and had significantly stronger correlation with IL-10, VEGF, IL-17, Tregs, MDSCs levels, NLR, WBC count and CRP in FBC, than in NFBC. In cases with high sIL-2R levels, Tregs and MDSCs levels were significantly higher and the overall survival (OS) and disease free survival (DFS) rates were significantly worse in FBC than in NFBC. Among the FBC cases with high sIL-2R levels, triple negative breast cancer tissues stained well for PD-L1 and CD8.

**Conclusions:** Compared with NFBC, FBC was associated with higher sIL-2R level, Th2 predominance, and less aggressive cancer immunosuppressive cells. In the present study, sIL-2R was identified as a biomarker that can predict the prognosis of FBC. The ability to prospectively identify patients who are less likely to have NFBC is a vital step in improving the overall survival of this population.

## Background

Familial breast cancer (FBC) is a cluster of breast cancer occurrence within a family. Most cases of breast cancer occur sporadically in individuals with little to no family history of the condition. Approximately 5% to 10% of breast cancer cases are considered hereditary through an autosomal dominant mechanism [1]. However, the diagnostic analysis of breast cancer-related genes, such as the *hereditary breast and ovarian cancer (HBOC)* gene, in patients and carriers, as well as the genetic testing of every single family member, remains very difficult to perform in a hospital setting. Furthermore, an increase in the prevalence of FBC is anticipated in the future. Regardless, two issues remain: the number of young breast cancer patients has increased, and patients with recurrent breast cancer require prompt treatment. Approximately 5-10% of breast cancers are considered “hereditary” and are thought to be caused by an inherited predisposition to breast cancer that is passed down through a family in an autosomal dominant manner. In some of these families, the underlying genetic cause is not known; however, many of these cases are caused by changes (mutations) in the *BRCA1*, *BRCA2*, *PTEN*, *TP53*, *CDH1*, or *STK11* genes (which are each associated with a unique hereditary cancer syndrome). Additional genes, such as *CHEK2*, *BRIP1*, *RAD51*, and *ATM*, are associated with breast and/or gynecologic cancers in some cases [1].

Interleukin (IL)-2, which is one of the most important cytokines for lymphocyte development, proliferation, and function, is produced by helper cells that differentiate from naïve cells on stimulation with interferon- $\gamma$  (IFN- $\gamma$ ) or IL-12. The actions of IL-2 include proliferation and activation of T cells, promotion of proliferation and antibody production of B cells, activation of monocytes/macrophages, and proliferation/activation of natural killer cells, to name a few [2]. In addition, IL-2 has been believed to be required for the maintenance of regulatory T cells (Tregs), which release the inhibitory cytokine IL-10 and exhibit immunosuppressive effects [3]. Breast cancer patients have been known to have increased blood levels of IL-2 and its soluble IL-2 receptor (sIL-2R) [4-7]. In this study, the role of sIL-2R was examined in patients with FBC.

*BRCA1/2* genes account for approximately 25% of all cases of FBC. Families with mutations in these genes usually have several members affected; carriers of mutations in *BRCA1* have a 70-80% chance of developing the disease [8, 9]. *BRCA1* is also involved in another type of DNA repair, termed mismatch repair. *BRCA1* interacts with the DNA mismatch repair protein MSH2 [10]. MSH2, MSH6, PARP, and some other proteins involved in single-strand repair are reported to be elevated in *BRCA1*-deficient mammary tumors [11]. A deficient DNA mismatch repair (MMR) function due to *MMR* gene mutation increases the number of somatic gene mutations and tumor mutational burden (TMB) and leads to the release of neoantigens from cancer cells that have a large number of gene mutations. Dendritic cells incorporate and degrade neoantigens, which are long peptides that bind to human leukocyte antigen (HLA) class II and are present on the cell surface. Neoantigens are recognized by naïve cluster of differentiation (CD) 4+ T cells and produce IL-2. The simultaneously incorporated and degraded short peptides bind to HLA class I present on the cell surface, are recognized by naïve CD8+ T cells, and express IL-2R. IL-2 binds to IL-2R to induce differentiation and proliferation of cytotoxic T lymphocytes (CTLs), which are cancer cell-specific immune cells. On activation, CTLs express immune checkpoint molecules, such as programmed cell death 1 (PD-1), on the surface. In addition, due to the IFN- $\gamma$  produced by CTLs for cancer cell attack, cancer cells express programmed cell death ligand 1 (PD-L1) and suppress CTL activity [12-14]. PD-L1 is a surface molecule that is expressed on different types of cells, including antigen-presenting cells, vascular endothelial cells, and other cells of human tissues. Expression of PD-L1 is also found on human tumor cells [15]. It is said that there are associations of *BRCA1*- and *BRCA2*-deficiency with features of genomic instability, expression of PD-L1 and PD-1, the landscape of inferred tumor-infiltrating immune cells, and the T-cell inflamed signature in breast cancers. It has been reported that *BRCA1*- and *BRCA2*-deficient breast cancers were associated with features of genomic instability, including increased mutation burden. Interestingly, *BRCA1*-deficient, but not *BRCA2*-deficient, breast cancers were associated with increased expression of PD-L1 and PD-1, higher abundance of tumor-infiltrating immune cells, and enrichment of the T cell-inflamed signature. The differences in immunophenotype between *BRCA1*- and *BRCA2*-deficient breast cancers can be attributed, in part, to *PTEN* gene mutation [16].

Eventually, CTLs become immune tolerant and incapable of functioning. In such cases, identification of the genes associated with increased TMB would enable the prediction of sensitivity to immune checkpoint inhibitors and more efficacious therapy for FBC. In the present study, the levels of sIL-2R, IL-10, vascular endothelial growth factor (VEGF), IL-17, immunosuppressive Tregs, myeloid derived suppressor cells (MDSCs), neutrophil to lymphocyte ratio (NLR), white blood cell (WBC) and C-reactive protein (CRP) levels were measured and compared between FBC and non-familial breast cancer (NFBC). Furthermore, CD8+ and PD-L1 staining of tissues was evaluated and compared between FBC and NFBC.

## Methods

### Study subjects

The present study enrolled 11 healthy volunteers and 106 patients who had histologically confirmed breast cancer and were treated at the Department of Breast Cancer Surgery of Fukushima Medical University (Fukushima, Japan) between January 2011 and June 2016. Staging was done in accordance with the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology: Breast Cancer Screening and Diagnosis [17, 18].

FBC was defined as a proband with breast cancer and (1) histologically proven breast cancer or ovarian cancer in at least 3 relatives, 1 of whom should be a 1<sup>st</sup>-degree relative, including the mother or a sister, and with bilateral breast cancer, prostate cancer, and pancreatic cancer in the family or (2) at least 2 successive generations diagnosed as having breast or ovarian cancer in at least 1 of the relatives at an age younger than 45 years and with bilateral breast cancer, prostate cancer, and pancreatic cancer in the family. NFBC was defined as only the proband having breast cancer.

After collection of blood samples from the study population, a total of  $1 \times 10^6$  peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll density gradient centrifugation method; aliquots of PBMCs were cryopreserved in a freezing medium. Plasma was separated by centrifugation and stored at  $-80^\circ\text{C}$  until flow cytometry analysis.

### **Cytokine production**

The serum concentrations of sIL-2R, IL-10, VEGF, and IL-17 in the supernatants were measured using an enzyme-linked immunosorbent assay kit (Quantikine; R&D Systems, Inc., Minneapolis, MN, USA), according to the manufacturer's protocol.

### **Flow cytometry analysis of Tregs**

A 3-color flow cytometry analysis was performed using a mixture of antibodies, including fluorescent isothiocyanate (FITC)-conjugated antihuman CD4, phycoerythrin-conjugated antihuman FoxP3, and allophycocyanin-conjugated antihuman CD25. Data acquisition and analysis were performed on a FACS Aria II flow cytometer (BD Biosciences, San Jose, CA, USA) using Flow Jo v10.2 software (Becton, Dickinson and Co., Becton Drive, Franklin Lakes, NJ, USA). The percentage of Tregs was calculated as a fraction of the total number of PBMCs.

### **Flow cytometry analysis of MDSCs**

Three-color flow cytometry analysis was performed with a mixture of antibodies, including FITC-conjugated antiCD14, phycoerythrin-conjugated antiCD11b, and phycoerythrin cyanin 5.1-conjugated antiCD33. Data acquisition and analysis were performed on a FACS Aria II flow cytometer using Flow Jo v10.2 software. The percentage of MDSCs was calculated as a fraction of the total number of PBMCs.

### **Statistical analysis**

All data are presented as means  $\pm$  standard deviation. Differences between groups were determined using Student's *t* test. Relationships between 2 variables were quantified using Spearman's rank correlation coefficient. For the assessment of overall survival (OS) and disease-free survival (DFS), data that were available until the last follow-up date or at 2,500 days were censored. The prognoses of the patients were analyzed using the Kaplan-Meier method, and the log-rank test was used to determine the significance of differences. Multivariate Cox regression analysis of the survival of patients with preoperative breast cancer was performed, according to tumor subtype and Ki67 status, as defined in the NCCN Clinical Practice Guidelines in Oncology [17, 18]. A Cox proportional hazards model was used to examine the simultaneous effects of multiple covariates on survival. The effect of each variable was described by

the hazard ratio with its 95% confidence interval. A  $p$  value of  $<0.05$  was considered to indicate significance. SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

### Staining for PD-L1 and CD8

Slides were deparaffinized in toluene, rehydrated in graded alcohols, and then heat-induced epitope retrieval was performed, followed by a CD8 IHC protocol on clone (C8/144B) Monoclonal Mouse (Dako, Agilent Technologies, Santa Clara, CA, USA) and a PD-L1 IHC protocol on clone E1L3N Rabbit (Cell Signaling Technology, Danvers, MA, USA).

## Results

The present study included a total of 106 patients; of these 24 had FBC and 82 had NFBC (Table 1). The median age of the patients was 42.0 years (range, 30-63 years) for FBC and 61.0 years (range, 46-88 years) for NFBC. The FBC group included 3 patients in stage I, 13 in stage II, 2 in stage III, and 6 in stage IV, whereas the NFBC group included 16 patients in stage I, 35 in stage II, 10 in stage III, and 21 in stage IV. None of the patients received anticancer treatment.

### Serum sIL-2R levels

As shown in Fig. 1, the sIL-2R levels were higher in the patients (FBC and NFBC,  $1014.2 \pm 79.2$  U/mL) than in the healthy volunteers ( $667.5 \pm 42.5$  U/mL). The sIL-2R level of FBC patients ( $1255.8 \pm 176.2$ ) was significantly higher than that of healthy volunteers ( $p=0.04$ ) and NFBC patients ( $865.4 \pm 58.8$  U/mL,  $p=0.01$ ).

### sIL-2R correlations

As shown in Fig. 2, the sIL-2R level of FBC patients had significant positive correlations with IL-10 production ( $r=0.48$ ,  $p=0.01$ ), the VEGF level ( $r=0.80$ ,  $p<0.0001$ ), IL-17 production ( $r=0.77$ ,  $p<0.0001$ ), the Treg level ( $r=0.80$ ,  $p<0.0001$ ), the MDSC level ( $r=0.70$ ,  $p=0.0002$ ), NLR ( $r=0.68$ ,  $p=0.0001$ ), WBC count ( $r=0.83$ ,  $p<0.0001$ ), and CRP level ( $r=0.64$ ,  $p=0.0009$ ).

The sIL-2R level was classified as high or low based on a cutoff value of 700 U/mL. The upper limit of 700 U/mL was chosen as the cutoff value for classifying sIL-2R as high or low based on the median sIL-2R value of  $667.50 \pm 42.54$  U/mL in healthy subjects. In FBC, the levels of Tregs ( $p=0.0008$ ) and MDSCs ( $p=0.01$ ) were more significantly increased with high serum sIL-2R levels than with low serum sIL-2R levels (Fig. 3).

### Overall and disease-free survival rates of FBC patients

As shown in Fig. 4, FBC patients who had high sIL-2R levels ( $\geq 700$  U/mL) had significantly worse OS ( $p=0.0091$ ) and DFS ( $p=0.0038$ ) rates compared with those with low sIL-2R levels.

The Kaplan-Meier plot of DFS was dichotomized, based on sIL-2R expression above and below the median value of 700 U/mL.-

### Immunohistochemistry of PD-L1 and CD8

As shown in Fig. 5, immunohistochemistry of the core needle biopsy specimens from FBC patients showed relatively more PD-L1 and CD8 when the sIL-2R level was high.

## Discussion

Breast cancer is a heterogeneous disease that comprises multiple molecular subtypes. In this study, there was no significant difference in tumor subtypes between FBC and NFBC patients (Table 1). Since Perou et al. used cDNA microarrays and performed gene expression profiling (GEP) of breast cancer in 2000, intrinsic subtype classification based on GEP has attracted attention [19, 20]. With this classification, breast cancer is divided into different subtypes of biologic properties such as luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like. Because the prognosis and drug sensitivity vary according to subtype, it could become the index for choosing pharmacotherapy, but performing GEP in all cases of breast cancer is not realistic in a clinic. Therefore, in the Sankt Gallen consensus meeting of 2011 and 2013, a substitute definition of intrinsic subtype based on the ER/PgR/HER2/Ki67 status, mainly composed of immunohistochemical examinations performed as part of common pathological examinations, was adopted [21-23].

For the definitions of subtypes, Table 1 includes ER-/PgR-/HER2- triple negative breast cancer (TNBC), ER-/PgR-/HER2+, ER+/PgR+/HER2+, ER+and/orPgR+/HER2-, Luminal A-like (high ER/PR and clearly low Ki-67 or grade), and Luminal B-like (lower ER/PR with a clearly high Ki-67, histological grade 3 in the clinical grouping) [24]. HER2-enriched and the basal-like subtype should be defined only by genetic analysis, but this is not done. In the latest report, the exact counting method performed with a light-microscope showed the predictive value of Ki-67 assessment with a 10% cut-off value [25]. In the present study, prognostic factors were evaluated with new subtype according to stage, but a prognostic factor with a significant difference was not identified because the number of FBC patients was small.

In several malignancies, serum sIL-2R levels are higher than in healthy individuals. Although sIL-2R is not organ-specific, except for malignant lymphoma, measuring its serum level was shown to be valuable for stage evaluation and monitoring during treatment [26]. Breast cancer patients have been shown to have increased serum levels of sIL-2R [27], as well as IL-10, VEGF, IL-17, Treg, and MDSC [28-32]. Others have reported the correlations of sIL-2R in cancer with IL-10, VEGF, and IL-17, and the relationship between Tregs and MDSCs [33-35].

Consistent with the previously reported results, the results of the present study showed increased sIL-2R levels in all breast cancer patients compared with levels in healthy subjects. Overall, sIL-2R level was significantly higher in FBC than in NFBC. Compared with NFBC, FBC comprises cancer cells that contain more mutations, release neoantigens and have CD8+ T cells in and around the microenvironment that express IL-2 receptors and release more sIL-2Rs in blood. Moreover, in FBC, there are simultaneous increases in the level of IL-10 and the number of anti-immunologic suppressor cells, such as Tregs and MDSCs. In the present study, sIL-2R level in FBC patients was significantly and positively correlated with IL-10, VEGF, IL-17, Tregs, and MDSCs. In particular, the significant positive correlation of the sIL-2R level with IL-10 indicated disruption of the Th2>Th1 balance, which leads to Th2 predominance and suppression of cellular immunity. The significant positive correlations of the sIL-2R level with VEGF and IL-17 suggested further tumor development. The high levels of both VEGF and IL-17 further increased the number of cancer immunosuppressive cells. On the other hand, the present results showed no significant, positive correlations of sIL-2R with IL-10, VEGF, IL-17, Tregs, or MDSCs in NFBC patients. These results suggested very more dramatic inflammatory and immune responses in the cancer microenvironment of FBC than of NFBC.

High sIL-2R significantly increased the Tregs and MDSCs in FBC patients, but not in NFBC patients. This result implies the importance of preventing the growth of cancer immunosuppressive cells, such as Tregs and MDSCs, in FBC patients. Moreover, high sIL-2R indicated a significantly worse prognosis in FBC than in NFBC. The elevated sIL-2R level may have predisposed the FBC patients to cancer growth rather than cancer suppression. Notably, proliferation of CTLs does not guarantee the removal of all cancer cells. IL-2 is a systemically administered treatment that can lead to serious side effects, but decreasing its dose may render it ineffective [36, 37].

PD-L1 is expressed in 20% of TNBCs, suggesting PD-L1 as a therapeutic target in TNBCs. Because PTEN loss is one mechanism regulating PD-L1 expression, agents targeting the PI3K pathway may increase the antitumor adaptive immune responses [38]. In this study, immunohistochemical analysis confirmed tissue expressions of PD-L1 and CD8 in FBC with high sIL-2R and less CD8 staining in NFBC. These results indicated similar expressions of PD-L1 in both FBC and NFBC, but, in the cancer microenvironment of FBC, Th2>Th1 balance occurred, because NLR, WBC, CRP, and IL-10, which are indices of inflammation, showed positive correlations. Furthermore, Tregs and MDSCs as anti-immunologic suppressor cells were present. Therefore, in and around the microenvironment, more CD8+ T cells seems to be induced and developed to attack a cancer. Although the direct relationship between IL-2 and PD-L1 is unknown, a combination of IL-2 and PD-L1 inhibitors has been the treatment regimen for chronic infections and cancer [39]. The duration of the proliferative contact between CD8+ T cells and antigen-presenting cells is affected by the sensitivity of individual CD8+ T cells to activation signals and by the concentration of IL-2 in the extracellular environment [40]. Regardless of the level of Tregs or MDSCs, immune checkpoint inhibitors should be effective, as long as CD8+ T cells proliferate. In the treatment of FBC patients, we should take into account the relationships between cancer and immunity, as well between cancer and genetics. In the future, shifting the Th1/Th2 balance towards Th1-dominant to inactivate cancer immunosuppressive cells may enhance the treatment efficacy of immune checkpoint inhibitors for patients with FBC.

## Conclusions

In the present study, sIL-2R appeared to be a reliable marker in FBC. The suppression of cellular immunity and increased number of cancer immunosuppressive cells brought about by high sIL-2R may be related to immunologic mechanisms. The possibility of targeting IL-2R expression and immune checkpoints needs to be investigated in future research.

## Abbreviations

**FBC:** familial breast cancer

**IL:** interleukin

**CTLs:** cytotoxic T lymphocytes

**sIL-2R:** soluble interleukin-2 receptor

**NFBC:** non-familial breast cancer

**VEGF:** vascular endothelial growth factor

**Tregs:** regulatory T cells

**MDSC:** myeloid derived suppressor cell

**NLR:** neutrophil to lymphocyte ratio

**WBC:** white blood cell

**CRP:** C-reactive protein

**PD-L1:** programmed cell death ligand 1

**CD8:** cluster of differentiation 8

**OS:** overall survival

**DFS:** disease free survival

**HBOC:** hereditary breast and ovarian cancer

**IFN- $\gamma$ :** interferon  $\gamma$

**MMR:** mismatch repair gene

**TMB:** tumor mutational burden

**HLA:** human leukocyte antigen

**PD-1:** programmed cell death 1

**PBMCs:** peripheral blood mononuclear cells

**GEP:** gene expression profiling

**TNBC:** triple negative breast cancer

## Declarations

### Ethics approval and consent to participate

The institutional review board and the local ethics committee (Ethical Review Board of Fukushima Medical University, 2011–2016) approved the study. The name of the study for the Fukushima Medical University IRB was “The study of immune suppression, inflammation, and nutritional damage in cancer patients.” Written, informed consent was obtained from all enrolled patients and healthy donors. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation at Fukushima Medical University and with the Helsinki Declaration of 1975, as revised in 2000. Each author certifies that all investigations were conducted in conformity with the ethical principles.

### Consent for publication

Written informed consent for the publication of this case report and any accompanying images was obtained from all patients. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

### Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no financial or nonfinancial competing interests.

### Funding

There is no funding to declare.

### Authors' contributions

KG performed patient recruitment and clinical investigation. KG, SH, YM, KS, and ST conceived and designed the study and drafted the manuscript. All authors read and approved the final manuscript.

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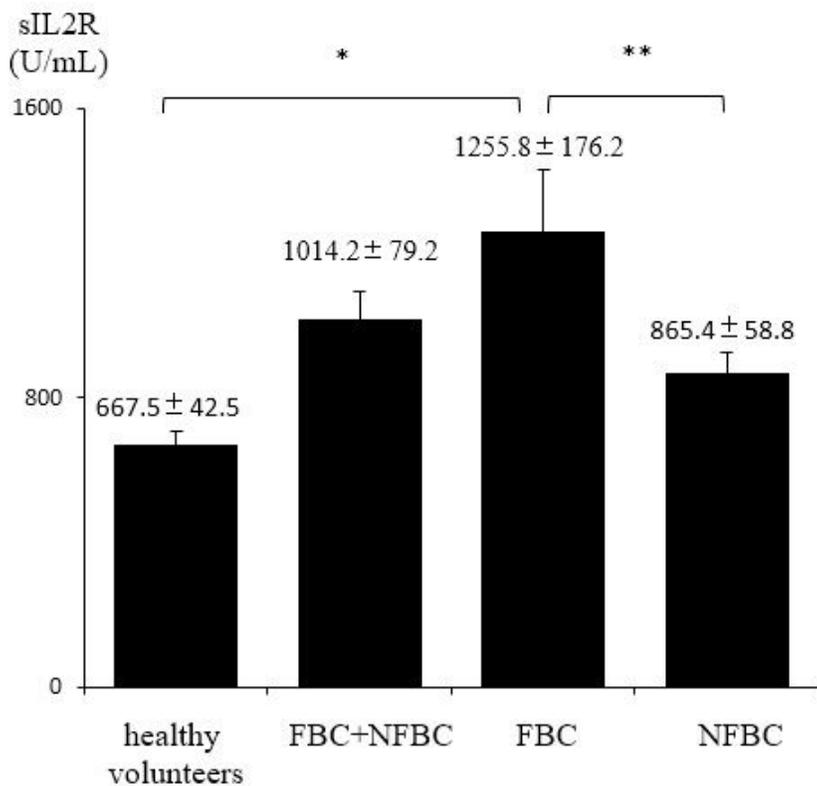
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## Table 1

Table 1. Clinical features of patients by subtype

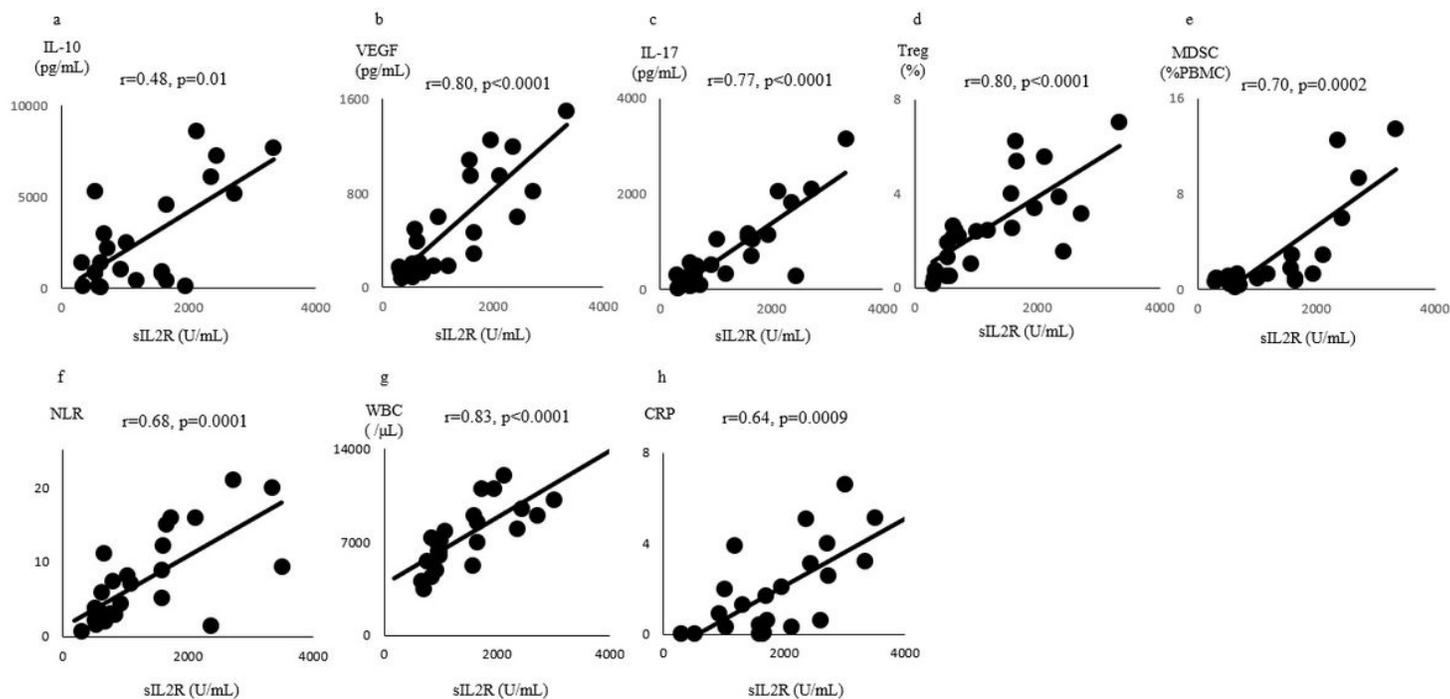
Stage	FBC (n=24)				NFBC (n=82)				
	I (n=3)	II (n=13)	III (n=2)	IV (n=6)	I (n=16)	II (n=35)	III (n=10)	IV (n=21)	
ER- / PgR- / HER2- (TNBC)	0	0	1	1	1	4	2	5	p<0.10
ER- / PgR- / HER2+	0	1	0	1	0	2	1	2	p<0.10
ER+ / PgR+ / HER2+	0	1	0	0	1	2	1	0	p<0.10
ER+ and/or PgR+ / HER2-	0	1	0	1	5	5	0	1	p<0.10
Luminal A-like	2	8	1	2	5	13	3	7	p<0.10
Luminal B-like	1	2	0	1	4	9	3	6	p<0.10

## Figures



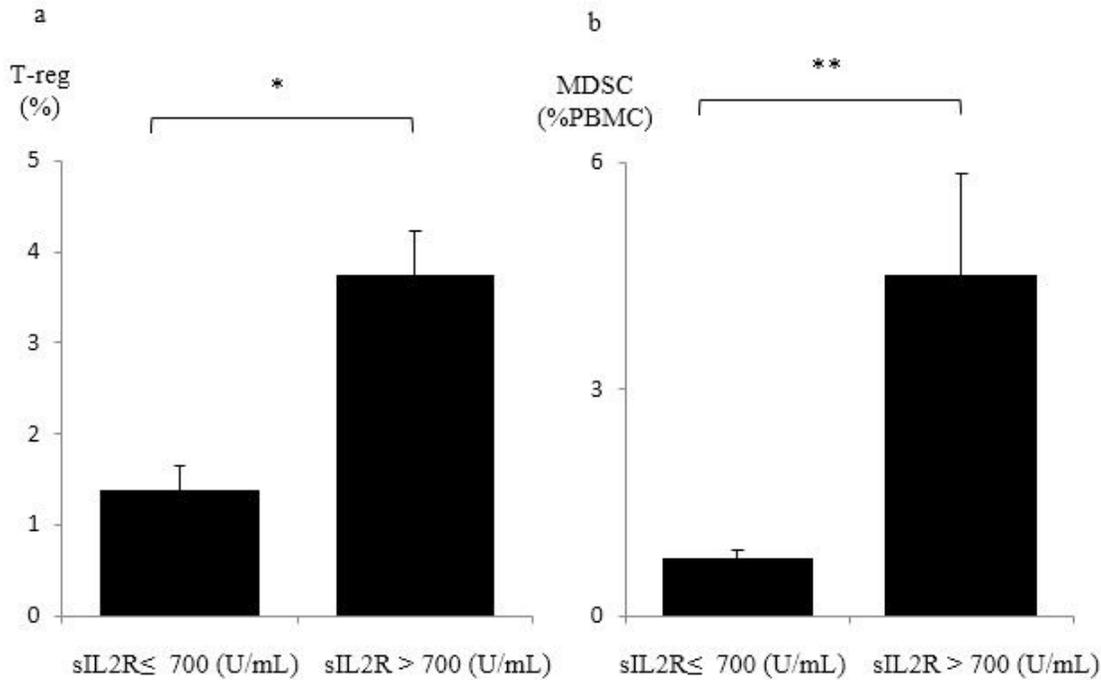
**Figure 1**

Results of sIL-2R evaluation in healthy volunteers and in patients with FBC + NFBC, FBC alone, and NFBC. The sIL-2R levels were higher in the preoperative patients (FBC + NFBC) than in the healthy volunteers. The sIL-2R level of FBC patients was significantly higher, compared with that of healthy volunteers (\* $p=0.0414$ ) and NFBC patients (\*\* $p=0.0155$ ). Data are represented as mean  $\pm$  SD. P values were determined using the Student's t-test.



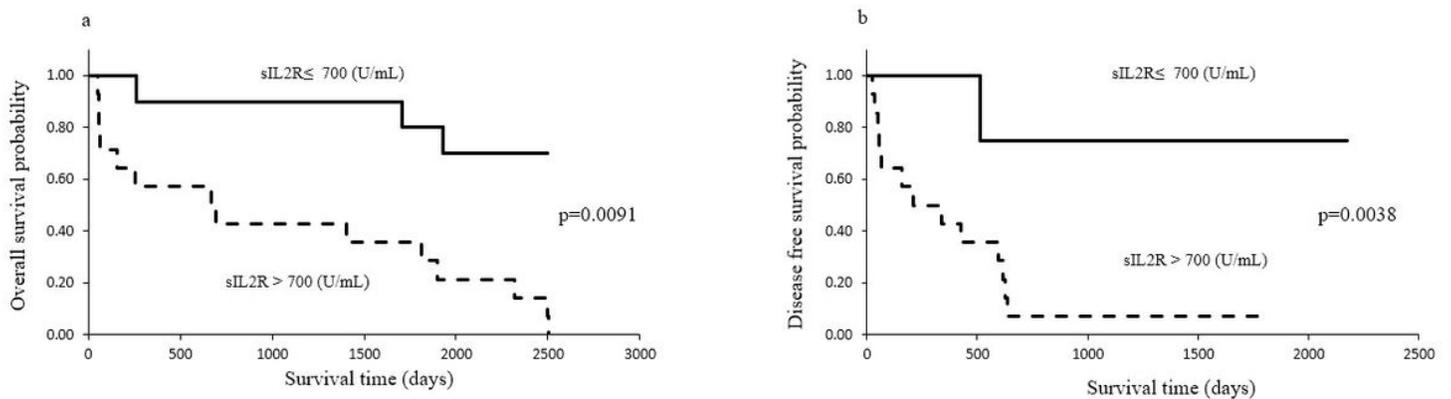
**Figure 2**

Correlations of sIL-2R with IL-10, VEGF, IL-17, Tregs, MDSCs, NLR, WBC, and CRP The sIL-2R levels of FBC patients are significantly positively correlated with (a) IL-10 production, (b) VEGF levels, (c) IL-17 production, (d) Treg levels, (e) MDSC levels, (f) NLR, (g) WBC count, and (h) CRP. The relationship between 2 variables was quantified by Spearman's rank correlation coefficient. IL-10: Interleukin-10, VEGF: vascular endothelial growth factor, IL-17: Interleukin-17, Treg: regulatory T cell, MDSC: myeloid derived suppressor cell, NLR: neutrophil to lymphocyte ratio, WBC: White blood cell, CRP: C-reactive protein



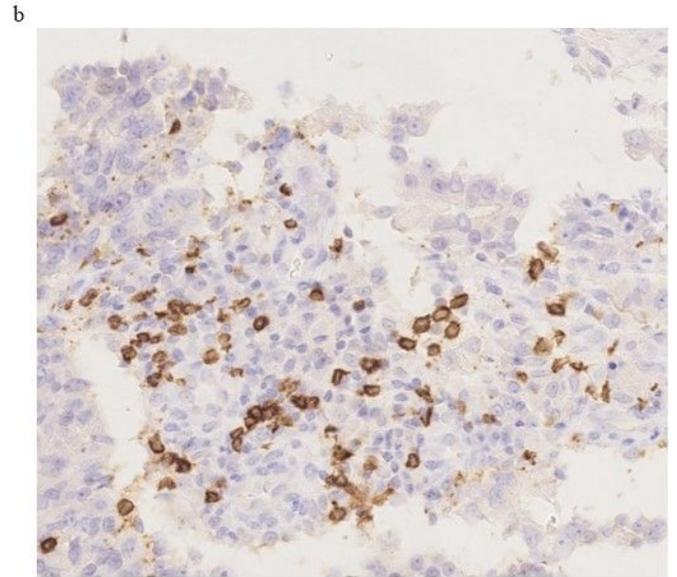
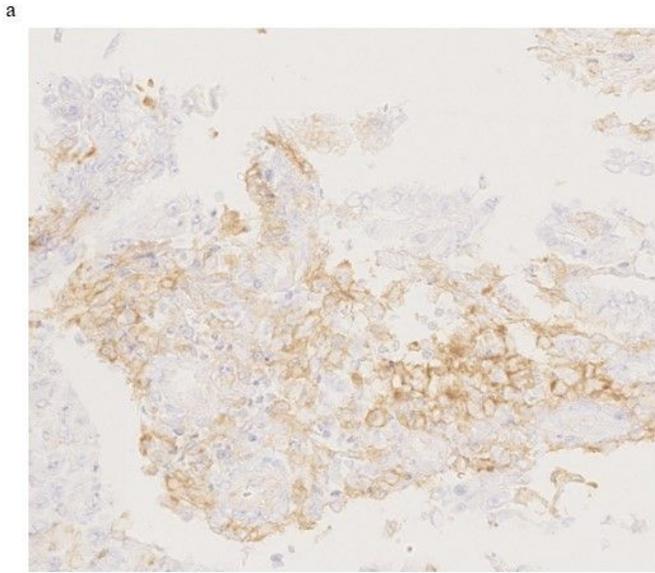
**Figure 3**

Tregs and MDSC levels according to the sIL-2R level in FBC patients. Based on a cutoff value of 700 U/mL, the levels of (a) Tregs (\* $p=0.0008$ ) and (b) MDSC (\*\* $p=0.01$ ) are significantly higher in patients with high sIL-2R than in those with low sIL-2R. Data are represented as mean  $\pm$  SD. P values were determined using the Student's t-test.



**Figure 4**

Kaplan–Meier estimates of overall and disease-free survival rates, according to surveillance status among FBC cases. The (a) OS and (b) DFS rates of FBC patients are significantly worse with high levels of sIL-2R (>700 U/mL) than with low levels of sIL-2R (<700 U/mL).



### Figure 5

Representative images of immunohistochemistry for PD-L1 and CD8. (a) The epithelial compartment is positive for PD-L1 ( $\times 10$ ), whereas (b) the infiltrating immune cells are positive for CD8 ( $\times 10$ ).