**Supplemental table 1.**

**Patient clinical information\***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Primary Mass Size (cm)** | **Histology** | **Grade** | **Lymph Node Involvement** | **Distant Metastasis** | **Stage** | **ER** | **PR** | **HER2** |
| **TB-005** | 1.5 | IDC |  Int | - | - | IIA | + | + | - |
| **TB-006** | 1.1 | IDC | Int | - | - | I | + | + | - |
| **TB-015** | 4.8 | IDC | Int | - | - | I | + | + | - |
| **TB-021** | 1.1 | IDC | High | - | - | I | - | - | - |
| **TB-035** | R:2.0L:2.5 | ILC | Int | - | - | II | + | + | - |
| **TB-039** | 1.5 | IDC | Int | - | - | I | + | + | + |
| **TB-043** | 2.2 | ILC |  Int | - | - | IIA | + | + | + |
| **TB-045** | 1.3 | IDC | High | - | - | IIA | + | + | - |
| **TB-047** | 0.6 | IDC | Low | - | - | I | + | + | - |
| **TB-049** | 1.4 | ILC |  Int | - | - | I | + | + | - |
| **TB-050** | 2.5 | IDC | Int | - | - | IIA | + | + | - |
| **TB-053** | 1.7 | IDC | High | - | - | I | + | + | - |
| **TB-054** | 2.2 | Mucinous |  Int | - | - | II | + | + | - |
| **TB-063** | 1.6 | IDC | Int | + | - | IIA | + | + | - |
| **TB-064** | 1.0 | IDC | Int | - | - | I | + | - | + |
| **TB-069** | 3.0 | IDC | Int | - | - | IIA | - | - | - |
| **TB-070** | 0.3 | ILC | Int | - | - | I | + | + | - |
| **TB-080** | 1.3 | IDC | High | - | - | I | - | - | + |
| **TB-092** | 1.7 | IDC | High | - | - | IA | - | - | + |
| **TB-095** | 2.0 | IDC | High | - | - | IIB | - | - | + |
| **TB-097** | 2.5 | IDC | Low | - | - | IIA | + | + | - |
| **TB-098** | 5.0 | ILC |  Int | - | - | III A | + | + | - |
| **TB-100** | 1.1 | ILC | Int | - | - | I | + | + | - |
| **TB-107** | 1.0 | IDC | High | - | - | IIA | + | + | - |
| **TB-108** | 2.5 | IDC | Int | - | - | IIA | + | + | + |
| ***TB-113*** | 5.0 | IDC | low | - | - | IIA | + | + | - |
| **TB-114** | 2.5 | IDC | High | - | - | IIA | - | - | - |
| **TB-120** | 1.7 | IDC | Int | - | - | IA | + | + | - |
| **TB-121** | 8.0 | ILC | Int | - | - | IIB | - | + | - |
| **TB-129** | 3.2 | IDC | High | - | - | IIA | - | - | - |
| **TB-131** | 0.9 | IDC | High | - | - | I | - | - | - |
| **TB-132** | 6.0 | IDC | High | - | - | III | - | - | - |
| **TB-134** | 0.8 | IDC | High | - | - | IB | - | - | - |
| **TB-135** | 1.4 | IDC | Low | - | - | IIA | + | + | - |
| **TB-136** | 1.0 | IDC | Int | - | - | IB | + | + | - |
| **TB-137** | 2.9 | IDC | Int | - | - | IIA | + | + | - |
| **TB-138** | 10.0 | ILC | Int | - | - | IIA | + | + | - |
| **TB 141** | 4.50.2 | IDC | High | - | - | IIB | - | - | - |
| **TB 144** | 2.1 | ILC | Int | - | - | IIA | + | + | - |
| **TB-146** | 1.5 | ILC | Low | - | - | I | + | - | - |
| **TB-147** | 1.8 | IDC | Int | - | - | I | + | + | - |
| **TB-150** | 2.5 | IDC | High | - | - | IIB | + | + | - |
| **TB-155** | 1.7 | IDC | Int | - | - | IA | + | + | - |
| **TB-158** | 1.9 | IDC | Low | - | - | IIB | + | + | - |
| **TB-159** | 1.9 | IDC | Int | + | - | IIIA | + | + | - |

\*Patients were enrolled at the hospital of the University of Texas Medical School at Houston according to the IRB approved protocol HSC-MS-11-0559. IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Int, intermedia grade; ER, estrogen receptor; PR, progesterone receptor.

**Supplemental table 2**

**Patient clinical information\***

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **Primary Mass Size (cm)** | **Histology** | **Grade** | **Lymph Node Involvement** | **Distant Metastasis** | **Stage** | **ER** | **PR** | **Her2** |
| P004 | 1.6 | IDC | High | + | - | II a | - | - | + |
| P013 | 3.6 | ILC | Low | + | - | IIIa | - | - | - |
| P017 | 2.1 | IDC | High | - | - | I a | - | - | - |
| P019 | 1.5 | IDC | High | - | - | II a | - | - | - |
| P020 | 2.8 | ILC | Int | - | - | II b | + | + | - |
| P022 | 2.5 | IDC | Int-High | - | - | IIa | + | + | - |
| P025 | 2.0 | IDC | Int | - | + | IV | + | + | - |
| P026 | 3.0 | IDC | High | - | - | IIa | - | - | - |
| P027 | 1.1 | IDC | Int | - | - | Ia | + | - | + |
| P028 | 5.3 | IDC | High | - | - | IIb | - | - | - |
| P029 | 2.0 | IDC | Low | - | - | Ia | + | + | - |
| P032 | 2.0 | ILC | High | - | - | IIa | + | + | - |
| P035 | 2.6 | IDC | Int | - | - | IIa | - | - | + |
| P039 | 4.0 | IDC | Int | + | - | IIIa | + | + | - |
| P041 | 2.5 | ILC | Int | - | - | IIa | + | + | - |

\*Patients were enrolled at the hospital of the University of Texas Medical School at Houston according to the IRB approved protocol HSC-MS-10-0580. IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Int, intermedia grade; ER, estrogen receptor; PR, progesterone receptor.



**Supplemental Figure 1. Standard curves developed for calculation of IgG subclasses.** The standard curves were generated using an ELISA kit (Invitrogen), A-D for IgG1-4, respectively. Both linear regression formula and R-square are listed in the graph for each subclass calculations. The standard curves were developed in each assay to reduce variations between experimental repeats.



**Supplemental Figure 2. Profiles of IgG subclasses in tumor and plasma samples.** Tumor tissues (**A**) showed lower IgG1 subclass than that in the paired plasma (**B**) in breast cancer patients. Percentage of each subclass is calculated based on the sum of all IgGs (IgG1 + IgG2 + IgG3 + IgG4).



**Supplemental Figure 3. IgG2 subclass showed much lower binding to Fc gamma receptors than the IgG1 counterpart.** Fc gamma receptor bindings of 4 monoclonal antibodies made in either IgG1or IgG2 subclasses by ELISA. (**A**) for FcγRI, (**B**) for FcγRII, and (**C**) for FcγRIII, respectively. Fc gamma receptors were coated on a high binding 96-well plate and antibody was added at the same concentration of 1 µg/ml, n=3.



**Supplemental Figure 4. Elevated scIgGs in tumor tissues were associated with reduced interactions with Fc gamma receptors.** (A) ScIgG1 trastuzumab (scIgG-T) showed reduced binding to Fc gamma receptors than the intact IgG1 (IgG-T, trastuzumab) counterpart. Y axis indicates the relative fluorescence units (RFU) by ELISA as described in the method. Error bars show standard deviation (SD) among three repeats (n=3). (B) IgGs in tumor lysates (Tumor) had decreased interactions with Fc gamma receptors compared to IgGs prepared from normal breast tissue lysates (Normal), n=15. ELISA was conducted by coating FcγRs on high binding 96-well plates, incubating with 10 µg IgGs/well, and detecting binding using an alkaline phosphatase-conjugated goat F(ab')2 fragment vs. human F(ab')2. The *p* values are 0.2688 and 0.1712 for FcγRII and FcγRIII, respectively. (C) Antibody IgGs enriched from tumor tissues (T-IgGs) showed decreased immune cell engagement in comparison with the same amount of antibodies enriched from normal breast tissues (N-IgGs) by flow analysis. Flow histograms of a representative sample with a mean fluorescence intensity (MFI) close to the average of the tumor group (T-IgGs) and of the normal breast tissue (N-IgGs) group, n=5. (D) Mean fluorescence intensities (MFI) (Y-axis) and standard deviations (SD) measured in flow analysis for IgGs extracted from patient tumor tissues (T-IgGs) in comparison with that from normal breast tissues (N-IgGs) are plotted in the bar graph, n=5.

**Supplemental Figure 5. Breast tumor tissues had significantly higher sum total of MMPs (MMP-1, 2 , 3, 8, 9, 10, 13) than in normal breast tissues**. (**A**) There was significantly higher MMPs (sum of 7 MMPs, Y-axis) in tumor tissues than that in normal tissues, n=25 for tumor samples and n=6 for normal breast tissue. (**B**) Levels of MMP-9 were the highest among the MMPs for the majority of tumor tissues from breast cancer patients. (**C**) Zymograph of MMP-9 using gelatin containing gel for MMP-9 activity detection. Cancer cell line BT474 (vehicle control) was used to express MMP-9 using a retro-vector expression system, and two stable clones, MMP-9 (1) and (2), showed expression of MMP-9, indicated by the arrow on the right side of the image.

**Supplemental Figure 6. There was no clear correlation between scIgG levels in tumor tissues and patient cancer grades or stages at the time of diagnosis**. (**A**) Levels of scIgGs (Y-axis) in tumor tissues from different cancer grades, high vs. low to intermediate grade (Int-Low). (**B**) Levels of scIgGs in tumor tissues from different cancer stages.