Use of Tumor-infiltrating lymphocytes (TILs) to predict the treatment response to eribulin chemotherapy in breast cancer

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Abstract

Background
Eribulin mesylate (eribulin) is currently indicated for treatment of locally advanced or metastatic breast cancer (MBC). It is a cytotoxic agent with unique mechanisms that suppress epithelial-mesenchymal transition (EMT) of cancer cells. On the other hand, Tumor-infiltrating lymphocytes (TILs), which are considered indicators of immune response monitoring, have been reported as prognostic factors and predictors of therapeutic efficacy. We thought that eribulin, which has an EMT-inhibiting mechanism, may produce an antitumor effect by improving the immune microenvironment, and in this study investigated the effects of breast cancer eribulin chemotherapy on the immune microenvironment with TILs as a marker.

Methods
TILs was evaluated in 52 patients with MBC who underwent chemotherapy with eribulin. The correlation between TILs evaluated according to the standard method, and prognosis, including the efficacy of eribulin chemotherapy, was investigated retrospectively.

Results
Of the 52 MBC patients, 29 (55.8%) were in the high TILs group and 23 (44.2%) were in the low TILs group. The high TILs group included significantly more triple-negative breast cancer (TNBC) (p = 0.008) than the low TILs group. In an analysis of outcomes, TNBC patients in the high TILs group had significantly longer disease-free survival than TNBC patients in the low TILs group (p = 0.033, log-rank), but no significant differences were seen in all breast cancer patients (p = 0.489, log-rank) or in non-TNBC patients (p = 0.878, log-rank). In a multivariate analysis of recurrence in TNBC patients, being in the high TILs group was again an independent factor for a good outcome (p = 0.031, HR = 0.063).
Conclusion
The results of this study suggest that TILs may be useful as a predictive marker of the therapeutic effect of eribulin chemotherapy in TNBC.

Introduction
Eribulin mesylate (eribulin) stops cell division by inhibiting microtubule extension [1–3], and has a mechanism of action that differs from other antimitotic drugs such as taxane and vinca alkaloids [2, 4, 5]. Thus, eribulin binds to microtubule ends and suppresses microtubule polymerization. Taxane binds extensively inside microtubules and suppresses shortening of microtubules by depolymerization. Vinca alkaloids bind to the external surface of microtubules and suppress both microtubule polymerization and depolymerization. Consequently, the anti-cancer effect differs among these agents. For example, in a phase III trial of eribulin (EMBRACE, Eisai Metastatic Breast Cancer Study Assessing Physician’s Choice versus E7389), a significant prolongation of overall survival was observed in patients with locally advanced or metastatic breast cancer (MBC) after eribulin treatment even without an improvement in disease free survival [6]. This effect was partially explained by a decrease in the occurrence of new metastatic lesions with eribulin therapy, an effect that has not been demonstrated with other drugs. However, the precise mechanism of this clinically significant benefit has not yet been elucidated. Some of the unique anticancer effects of eribulin have emerged from experimental studies using cancer cells and tumor tissues [7, 8]. These include suppression of the epithelial-mesenchymal transition (EMT) of cancer cells and promotion of vascular remodeling in tumors.

Stephen Paget proposed the “seed and soil” theory with regard to cancer metastases in 1889, and, since that time, the importance of the tumor microenvironment for cancer cell proliferation has been increasingly recognized [9, 10]. Tumor tissue is composed not only of cancer cells, but also inflammatory cells, immunocytes, vascular and lymphatic cells, fibroblasts, and fibrous tissue, and these elements comprise the characteristic tumor microenvironment. The importance of regulating and improving the immune microenvironment in cancer has been recognized because the immune microenvironment in cancer tissues affects not only the efficacy of immunotherapy, but also the efficacy and prognosis of conventional chemotherapy and other modes of anticancer therapy [11, 12]. Therefore, monitoring the host’s immune response to cancer in the microenvironment is believed to play a key role in predicting therapeutic efficacy and prognosis. Tumor-infiltrating lymphocytes (TILs), which are considered indicators of immune response monitoring, have been reported as prognostic factors and predictors of therapeutic efficacy [13–15].

The progression of cancer is not determined solely by the properties of the cancer cells themselves; it is also closely associated with the interrelation between cancer cells and their microenvironment, including EMT and immune responses. EMT suppression seems to contribute to improving the immune microenvironment [16]. We therefore thought that eribulin, which has an EMT-inhibiting mechanism, may produce an antitumor effect by improving the immune microenvironment, and in this study investigated the effects of breast cancer eribulin chemotherapy on the immune microenvironment with TILs as a marker.

Materials and methods
Patient background
The subjects included 52 patients with MBC who underwent chemotherapy using eribulin from August 2011 to June 2013 at our institute. The median follow-up time was 431 days.
(range, 50–650 days). The overall response rate (ORR), clinical benefit rate (CBR), disease control rate (DCR), overall survival (OS), time to treatment failure (TTF) and progression-free survival (PFS) were calculated regarding the efficacy of this regimen. The TTF was evaluated on a daily basis and set as the period from the date of treatment commencement to cancellation for any reason, including disease aggravation, treatment toxicity and death. The OS was evaluated on a daily basis and set as the period from the date of treatment commencement to death. The PFS was evaluated on a daily basis and set as the period from the date of treatment commencement to either the earlier of the date of death or confirmation of progressive disease (PD).

Regarding the outline of the chemotherapy regimen, one course of treatment consisted of 21 days (three weeks). Eribulin mesylate (1.4 mg/m²) was intravenously administered on days 1 and 8, after which a withdrawal period was continued to day 21 [6]. This protocol was repeated until PD was detected or a severe adverse event requiring the discontinuation of the scheduled chemotherapy was noted. The chemotherapy was administered on an outpatient basis in all cases. The antitumor effect was evaluated based on the criteria for therapeutic effects conforming to the RECIST criteria (Response Evaluation Criteria in Solid Tumors) version 1.1 [17].

The morphology of the tumor, including the histological tissue type, nucleus grade, etc., was evaluated using conventional hematoxylin and eosin (HE) staining. Moreover, breast cancer was classified into subtypes according to the immunohistochemical expression of the estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) and Ki67. Based on their immunohistochemical expression, the tumours are categorized into the immunophenotypes luminal A (ER+ and/or PgR+, HER2-, Ki67-low), luminal B (ER+ and/or PgR+, HER2+) (ER+ and/or PgR+, HER2-, Ki67-high), HER2-enriched (ER-, PgR-, and HER2+), and triple-negative breast cancer (TNBC) (negative for ER, PgR and HER2) [18].

Ethics statement

The design of this study is a retrospective chart review study. Written informed consent was obtained from all subjects. This research conformed to the provisions of the Declaration of Helsinki in 2013. All patients were informed of the investigational nature of this study and provided their written, informed consent. The study protocol was approved by the Ethics Committee of Osaka City University (#926).

Histopathological evaluation

Histopathological assessment of predictive factors was made for core needle biopsy (CNB) specimens for primary lesions at the time of the breast cancer diagnosis. Histopathologic analysis of the percentage of TILs was evaluated on a single full-face hematoxylin and eosin (HE)-stained tumor section using criteria described by Salgado et al [19]. TILs were defined as the infiltrating lymphocytes within tumor stroma and were expressed in proportion to the field investigated [19–21]. The area of in situ carcinoma and crush artifacts were not included. Proportional scores were defined as 3, 2, 1, and 0 if the area of stroma with lymphoplasmacytic infiltration around invasive tumor cell nests was > 50%, > 10–50%, ≤ 10%, and absent, respectively (Fig 1). TILs were considered positive when scores were ≥ 2, and negative when scores were 1 and 0. Histopathologic evaluation of TILs was jointly performed by two breast pathologists, who were blinded to clinical information, including treatment allocation and outcomes.
Statistical analysis

Statistical analysis was performed using the SPSS® version 19.0 statistical software package (IBM, Armonk, NY, USA). Categorical data are reported with numbers and percentages, and continuous data as a median and range. The association between TILs and other clinicopathological variables, and the significance of different prognostic markers were analysed using the chi-squared test (or Fisher’s exact test when necessary). The association with survival was analysed using the Kaplan–Meier plot and log-rank test. The Cox proportional hazards model was used to compute univariate and multivariate hazard ratios (HR) for the study parameters with 95% confidence intervals (CI), and used in a backward stepwise method for variable selection in multivariate analysis. In all of the tests, a p-value of less than 0.05 was considered statistically significant. Cut-off values for different biomarkers included in this study were chosen before statistical analysis.

Results

Clinical effects of eribulin chemotherapy

The subjects included 52 patients who underwent chemotherapy using eribulin against MBC. The clinical effects were as follows: ORR = 34.6% (18/52); CBR = 44.2% (23/52); DCR = 51.9% (27/52); median OS = 334 days; median TTF = 81 days; and median PFS = 275 days. The distribution of the intrinsic subtype classification was as follows: Luminal A = 12 cases (23.1%); Luminal B = 13 cases (15.0%); Luminal HER2 = 2 cases (3.8%); HER2 enriched = 3 cases (5.8%) (non-TNBC 30 cases, 57.7%); and TNBC = 22 cases (42.3%). In an investigation according to the intrinsic subtype, the respective ORR was found to be 40.0% (12/30) in the non-TNBC cases and 27.3% (6/22) in the TNBC cases (S1 Table).

Tumor-infiltrating lymphocytes in eribulin chemotherapy cases

TILs were determined in every sample and ranged from 0 to 88 (mean, 15; median, 18; standard deviation 5). Of the 52 patients, 29 (55.8%) were in the high TILs group and 23 (44.2%) were in the low TILs group. The high TILs group included significantly more TNBC (p = 0.008) than the low TILs group, but no correlations were seen with any other clinicopathological factors (Table 1). TILs were not correlated with any clinicopathological factors in either TNBC or non-TNBC.

In an analysis of outcomes, TNBC patients in the high TILs group had significantly longer disease-free survival than TNBC patients in the low TILs group (p = 0.033, log-rank), but no significant differences were seen in all breast cancer patients (p = 0.489, log-rank) or in non-TNBC patients (p = 0.878, log-rank) (Fig 2A–2C). Similarly, among TNBC patients OS was significantly longer in the high TILs group than in the low TILs group (p = 0.042, log-rank) (Fig 3A–3C). However, no increase in OS was seen among all breast cancer patients (p = 0.668, log-rank) or among non-TNBC patients (p = 0.535, log-rank). With regard to TTF, no significant differences were seen in any subtype (Fig 3D–3F).

In a univariate analysis of recurrence in TNBC patients, being in the high TILs group was a factor for a good outcome (p = 0.047, HR = 0.260). In a multivariate analysis, being in the high TILs group was again an independent factor for a good outcome (p = 0.031, HR = 0.063) (Table 2).

Discussion

EMT is observed when cancer spreads, and promotes cancer infiltration and metastasis by facilitating the ability of cancer cells to move and the breakdown of the extracellular matrix [22]. Cancer cells with induced EMT are known to acquire treatment resistance and to have...
enhanced properties as cancer stem cells [23]. It is also reported that inhibiting EMT improves the cancer immune microenvironment and enhances the antitumor immune response [24]. An enhanced antitumor immune response contributes not only to immunotherapy but also to the antitumor effect of conventional chemotherapy [11]. Thus, inhibition of EMT with eribulin chemotherapy is thought to enhance the antitumor immune response via improvement in the cancer immune microenvironment.

Among the intrinsic subtypes of breast cancer, eribulin chemotherapy is also reported to be particularly useful for TNBC [25, 26]. In recent years it has been shown that TNBC can be subdivided into 7 different subtypes according to gene expression profile [27–29]. Among them are mesenchymal (M) and mesenchymal-stem like (MSL) subtypes that have high levels of expression of EMT-related genes (also high expression levels of stem cell-related genes). Eribulin plays a role in EMT inhibition, and seems promising as a drug that is effective against these subtypes of TNBC.

In this study, TILs were significantly higher in TNBC patients than in non-TNBC patients. High levels of TILs, a marker for monitoring the antitumor immune response, suggest a high level of immune activity in TNBC patients. In the TNBC subtype classification, there is an immunomodulatory (IM) subtype with high expression levels of genes related to immune response [27], and it may be that cases with high levels of TILs are related to these subtypes.

In an analysis of outcomes among TNBC patients, longer PFS and OS were seen in the high TILs group than in the low TILs group. The Kaplan-Meier curve in this investigation showed a
Table 1. Correlations between tumor-infiltrating lymphocytes and clinicopathological parameters in 52 locally advanced or metastatic breast cancers and their Triple negative- and non-Triple negative-subtypes.

| Parameters                  | All breast cancer (n = 52) |  |  |  |  |
|-----------------------------|---------------------------|--|--|--|--|
|                             | High (n = 29)             | Low (n = 23)  | p value | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Estrogen receptor           |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Negative                    | 16 (55.2%)                | 7 (30.4%)    | 0.074   | 9 (52.9%)     | 3 (60.0%)   | 0.594   | 8 (66.7%)     | 8 (44.4%)    | 0.206   |  |
| Positive                    | 13 (44.8%)                | 16 (69.6%)   |         | 8 (47.1%)     | 2 (40.0%)   |         | 8 (66.7%)     | 8 (44.4%)    |         |  |
| Progesterone receptor       |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Negative                    | 20 (69.0%)                | 12 (52.2%)   | 0.216   | 12 (70.6%)    | 4 (80.0%)   | 0.581   | 9 (75.0%)     | 14 (77.8%)   | 0.597   |  |
| Positive                    | 9 (31.0%)                 | 11 (47.8%)   |         | 8 (29.4%)     | 1 (20.0%)   |         | 1 (20.0%)     | 2 (20.0%)    |         |  |
| HER2                        |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Negative                    | 26 (89.7%)                | 21 (91.3%)   | 0.612   | 17 (76.5%)    | 4 (80.0%)   | 0.687   | 0 (0.0%)      | 2 (11.1%)    | 0.352   |  |
| Positive                    | 3 (10.3%)                 | 2 (8.7%)     |         | 9 (23.5%)     | 1 (20.0%)   |         | 12 (100.0%)   | 16 (88.9%)   |         |  |
| HR and HER2 status          |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| TNBC                        | 17 (58.6%)                | 5 (21.7%)    | 0.008   | 12 (41.4%)    | 18 (78.3%)  |         | 12 (41.4%)    | 18 (78.3%)   |         |  |
| non-TNBC                    | 12 (41.4%)                | 18 (78.3%)   |         | 12 (41.4%)    | 18 (78.3%)  |         | 12 (41.4%)    | 18 (78.3%)   |         |  |
| Age at chemotherapy         |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| ≤63                         | 13 (44.8%)                | 13 (56.5%)   | 0.402   | 9 (52.9%)     | 3 (60.0%)   | 0.594   | 8 (66.7%)     | 8 (44.4%)    | 0.206   |  |
| >63                         | 16 (55.2%)                | 10 (43.5%)   |         | 8 (47.1%)     | 2 (40.0%)   |         | 8 (66.7%)     | 8 (44.4%)    |         |  |
| Degree of progress          |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Locally advanced            | 8 (27.6%)                 | 5 (21.7%)    | 0.402   | 5 (29.4%)     | 1 (20.0%)   | 0.594   | 3 (25.0%)     | 4 (22.2%)    | 0.597   |  |
| Visceral metastases         | 21 (72.4%)                | 18 (78.3%)   | 0.629   | 12 (70.6%)    | 4 (80.0%)   | 0.581   | 9 (75.0%)     | 14 (77.8%)   | 0.597   |  |
| Life threatening condition  |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| non-Life threatening        | 21 (72.4%)                | 17 (73.9%)   | 0.904   | 6 (35.3%)     | 3 (60.0%)   | 0.316   | 2 (16.7%)     | 3 (16.7%)    | 0.696   |  |
| Life threatening            | 8 (27.6%)                 | 6 (26.1%)    |         | 6 (35.3%)     | 3 (60.0%)   |         | 10 (83.3%)    | 15 (83.3%)   |         |  |
| Nuclear grade               |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| 1, 2                        | 16 (55.2%)                | 17 (73.9%)   | 0.904   | 6 (35.3%)     | 3 (60.0%)   | 0.316   | 2 (16.7%)     | 3 (16.7%)    | 0.696   |  |
| 3                           | 13 (44.8%)                | 6 (26.1%)    | 0.163   | 13 (76.5%)    | 4 (80.0%)   | 0.687   | 0 (0.0%)      | 2 (11.1%)    | 0.352   |  |
| Ki67                        |                           |              |         | High (n = 17) | Low (n = 5) | p value | High (n = 12) | Low (n = 18) | p value |  |
| Negative                    | 13 (44.8%)                | 13 (56.5%)   | 0.163   | 7 (41.2%)     | 4 (80.0%)   | 0.687   | 0 (0.0%)      | 2 (11.1%)    | 0.352   |  |
| Positive                    | 16 (55.2%)                | 10 (43.5%)   |         | 10 (58.8%)    | 1 (20.0%)   | 0.155   | 6 (50.0%)     | 9 (50.0%)    | 0.645   |  |
| HR, hormone receptor. HER2, human epidermal growth factor receptor 2. TNBC, triple-negative breast cancer.

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Fig 2. In an analysis of outcomes, TNBC patients in the high TILs group had significantly longer disease-free survival than TNBC patients in the low TILs group (p = 0.033, log-rank) (A), but no significant differences were seen in all breast cancer patients (p = 0.489, log-rank) (B) or in non-TNBC patients (p = 0.878, log-rank) (C).

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characteristic delayed separation curve in immunotherapy in the high TILs group, and despite the short follow-up time, one may conjecture that eribulin chemotherapy contributes to the

Fig 3. Among TNBC patients OS was significantly longer in the high TILs group than in the low TILs group (p = 0.042, log-rank) (A). However, no increase in OS was seen among all breast cancer patients (p = 0.668, log-rank) (B) or among non-TNBC patients (p = 0.535, log-rank) (C). With regard to TTF, no significant differences were seen in any subtype (D–F).

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Table 2. Univariate and multivariate analysis with respect to progression free survival in 22 triple-negative breast cancers.

| Parameters                          | Univariate analysis | Multivariate analysis |
|-------------------------------------|---------------------|-----------------------|
|                                     | Hazard ratio 95% CI | p value               | Hazard ratio 95% CI | p value               |
| Age at chemotherapy                 | <63 vs >63          | 0.470 0.117–1.893     | 0.288                |
| Degree of progress                  | Locally advanced vs Visceral metastases | 1.109 0.230–5.352 | 0.898                |
| Life threatening condition          | non-Life threatening vs Life threatening | 1.720 0.460–6.427 | 0.420                |
| Nuclear grade                       | 1, 2, vs 3          | 2.915 0.364–23.352    | 0.314                | 2.045 0.208–20.120 0.540 |
| Ki67                                | ≤14% vs >14%        | 1.368 0.364–5.133     | 0.642                | 5.736 0.438–75.058 0.183 |
| TILs                                | High vs Low         | 0.260 0.069–0.980     | 0.047                | 0.063 0.005–0.771 0.031 |

CI, confidence interval. TILs, tumor-infiltrating lymphocytes.

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antitumor immune response. The enhanced antitumor immune response that accompanies EMT suppression with eribulin chemotherapy may be behind the longer OS in the EMBRACE trial [6, 26].

In breast cancer chemotherapy, TILs are thought to be useful as a marker to predict the therapeutic effect in TNBC and HER2-positive breast cancers [13–15, 30]. However, these reports are with anthracycline, taxanes, platinum-based agents, and trastuzumab; the clinical relevancy of eribulin and TILs has yet to be demonstrated. This study had a small number of patients, and, although the HER2-positive breast cancer data could not be confirmed, the study showed that in TNBC the antitumor immune response could be monitored with TILs. The ability to predict the therapeutic effect of eribulin chemotherapy with TILs would seem to be promising in that it could select only those patients who would respond to combination therapy with eribulin chemotherapy and immune therapy.

Conclusions
The results of this study suggest that TILs may be useful as a predictive marker of the therapeutic effect of eribulin chemotherapy in TNBC.

Supporting information
S1 Table. Clinical effects of eribulin chemotherapy in breast cancer subtypes. The clinical effects were as follows: overall ORR = 34.6% (18/52); CBR = 44.2% (23/52); DCR = 51.9% (27/52). In an investigation according to the intrinsic subtype, the respective ORR was found to be 40.0% (12/30) in the non-TNBC cases and 27.3% (6/22) in the TNBC cases.

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References
1. Hirata J, Katsuino M, Kaneko S, Umemura T, Nishimura J, Motomura S, et al. Clinical significance of human bone marrow stromal cell colonies in acute leukemias. Leuk Res. 1986; 10(12):1441–5. Epub 1986/01/01. PMID: 3796036
2. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004; 4(4):253–65. Epub 2004/04/02. doi: 10.1038/nrc1317 PMID: 15057285
3. Kuznetsov G, Towle MJ, Cheng H, Kawamura T, TenDyke K, Liu D, et al. Induction of morphological and biochemical apoptosis following prolonged mitotic blockage by halichondrin B macrocyclic ketone analog E7389. Cancer Res. 2004; 64(16):5760–6. Epub 2004/08/18. doi: 10.1158/0008-5472.CAN-04-1169 PMID: 15313917
4. Bai RL, Pauli KD, Herald CL, Malspeis L, Pettit GR, Hamele E. Halichondrin B and homohalichondrin B, marine natural products binding in the vinca domain of tubulin. Discovery of tubulin-based mechanism of action by analysis of differential cytotoxicity data. J Biol Chem. 1991; 266(24):15882–9. Epub 1991/08/25. PMID: 1874739
5. Ledford H. Complex synthesis yields breast-cancer therapy. Nature. 2010; 468(7324):608–9. Epub 2010/12/03. doi: 10.1038/468608a PMID: 21124423
6. Cortes J, O’Shaughnessy J, Loesch D, Blum JL, Vahdat LT, Petrakova K, et al. Eribulin monotherapy versus treatment of physician’s choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study. Lancet. 2011; 377(9768):914–23. Epub 2011/03/08. doi: 10.1016/S0140-6736(11)60070-6 PMID: 21376385
7. Yoshida T, Ozawa Y, Kimura T, Sato Y, Kuznetsov G, Xu S, et al. Eribulin mesilate suppresses experimental metastasis of breast cancer cells by reversing phenotype from epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET) states. Br J Cancer. 2014; 110(6):1497–505. Epub 2014/02/27. PubMed Central PMID: 24170852
8. Terashima M, Sakai K, Togashi Y, Hayashi H, De Velasco MA, Tsurutani J, et al. Synergistic antitumor effects of S-1 with eribulin in vitro and in vivo for triple-negative breast cancer cell lines. Springerplus. 2014; 3:417. Epub 2014/08/21. PubMed Central PMID: 25140293
9. Mathot L, Stenninger J. Behavior of seeds and soil in the mechanism of metastasis: a deeper understanding. Cancer Sci. 2012; 103(4):626–31. Epub 2012/01/04. doi: 10.1111/j.1349-7006.2011.02195.x PMID: 22128285
10. Fidler IJ. The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis revisited. Nat Rev Cancer. 2003; 3(6):453–8. Epub 2003/06/05. doi: 10.1038/nrc1098 PMID: 12778135
11. Fridman WH, Pages F, Sautes-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012; 12(4):298–306. doi: 10.1038/nrclinonc.2011.223 PMID: 21646868
12. Liu H, Zhang T, Ye J, Li H, Huang J, Li X, et al. Tumor-infiltrating lymphocytes predict response to chemotherapy in patients with advance non-small cell lung cancer. Cancer Immunol Immunother. 2012; 61 (10):1849–56. Epub 2012/03/30. doi: 10.1007/s00262-012-1231-7 PMID: 22458675
13. Kocian P, Sedivcova M, Drgac J, Cerna K, Hoch J, Kodel R, et al. Tumor-infiltrating lymphocytes and dendritic cells in human colorectal cancer: their relationship to KRAS mutational status and disease recurrence. Hum Immunol. 2011; 72(11):1022–8. doi: 10.1016/j.humimm.2011.07.012 PMID: 21864745
14. Lee WS, Kang M, Baek JH, Lee JI, Ha SY. Clinical impact of tumor-infiltrating lymphocytes for survival in curatively resected stage IV colon cancer with isolated liver or lung metastasis. Ann Surg Oncol. 2013; 20(2):697–702. doi: 10.1007/s10434-012-2752-1 PMID: 23224827
15. Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y. Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell. 2009; 15(3):195–206. Epub 2009/03/03. doi: 10.1016/j.ccr.2009.01.023 PMID: 19249678
16. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45(2):228–47. doi: 10.1016/ejca.2008.10.026 PMID: 19097774
18. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thurlimann B, Senn HJ. Strategies for subtypes—
dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus
on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol. 2011; 22(8):1736–47. Epub 2011/06/
29. PubMed Central PMCID: PMC3144634. doi: 10.1093/annonc/mdr304 PMID: 21709140

19. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-
infiltrating lymphocytes (TILs) in breast cancer; recommendations by an International TILs Working
Group 2014. Ann Oncol. 2015; 26(2):259–71. Epub 2014/09/13. doi: 10.1093/annonc/mdu450 PMID:
25214542

20. Ono M, Tsuda H, Shimizu C, Yamamoto S, Shibata T, Yamamoto H, et al. Tumor-infiltrating lympho-
cytes are correlated with response to neoadjuvant chemotherapy in triple-negative breast cancer.
Breast Cancer Res Treat. 2012; 132(3):793–805. doi: 10.1007/s10549-011-1554-7 PMID: 21562709

21. Mao Y, Qu Q, Zhang Y, Liu J, Chen X, Shen K. The value of tumor infiltrating lymphocytes (TILs) for pre-
dicting response to neoadjuvant chemotherapy in breast cancer: a systematic review and meta-analy-
sis. PLoS One. 2014; 9(12):e115103. PubMed Central PMCID: PMC4264870. doi: 10.1371/journal.
pone.0115103 PMID: 25501357

22. Thiery JP, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and
disease. Cell. 2009; 139(5):871–90. Epub 2009/12/01. doi: 10.1016/j.cell.2009.11.007 PMID:
19945376

23. Floor S, van Staveren WC, Larsimont D, Dumont JE, Maenhaut C. Cancer cells in epithelial-to-mesenchymal
transition and tumor-propagating-cancer stem cells: distinct, overlapping or same populations. Oncogene.
2011; 30(46):4809–21. Epub 2011/06/07. doi: 10.1038/onc.2011.184 PMID: 21843013

24. Jing Y, Han Z, Zhang S, Liu Y, Wei L. Epithelial-Mesenchymal Transition in tumor microenvironment.
Cell Biosci. 2011; 1:29. Epub 2011/09/02. 2045-3701-1-29 [pii]. PubMed Central PMCID: PMC3179439.
doi: 10.1186/2045-3701-1-29 PMID: 21880137

25. Kaufman PA, Awada A, Twelves C, Yelle L, Perez EA, Velikova G, et al. Phase III open-label random-
ized study of eribulin mesylate versus capecitabine in patients with locally advanced or metastatic
breast cancer previously treated with an anthracycline and a taxane. J Clin Oncol. 2015; 33(6):594–
601. Epub 2015/01/22. PubMed Central PMCID: PMC4463422. doi: 10.1200/JCO.2013.52.4892
PMID: 25605862

26. Twelves C, Cortes J, Vahdat L, Olivo M, He Y, Kaufman PA, et al. Erratum to: Efficacy of eribulin in
women with metastatic breast cancer: a pooled analysis of two phase 3 studies. Breast Cancer Res
Treat. 2015; 149(1):313. Epub 2015/01/13. doi: 10.1007/s10549-014-3245-7 PMID: 25573650

27. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human
triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin
Invest. 2011; 121(7):2750–67. PubMed Central PMCID: PMC3127435. doi: 10.1172/JCI45014 PMID:
21633166

28. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, et al. Differential
response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes.
Clin Cancer Res. 2013; 19(19):5533–40. PubMed Central PMCID: PMC3813597. doi: 10.1158/1078-
0432.CCR-13-0799 PMID: 23948975

29. Metzger-Filho O, Tutt A, de Azambuja E, Saini KS, Viale G, Loi S, et al. Dissecting the heterogeneity of
triple-negative breast cancer. J Clin Oncol. 2012; 30(15):1879–87. doi: 10.1200/JCO.2011.38.2010
PMID: 22454417

30. Loi S, Michiels S, Salgado R, Sirtaine N, Jose V, Fumagalli D, et al. Tumor infiltrating lymphocytes are
prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer:
results from the FinHER trial. Ann Oncol. 2014; 25(8):1544–50. doi: 10.1093/annonc/mdu112 PMID:
24608200