

Molecular Pathways: Involvement of Immune Pathways in the Therapeutic Response and Outcome in Breast Cancer

Fabrice Andre^{1,2}, Maria V. Dieci³, Peter Dubsy^{5,6}, Christos Sotiriou⁷, Giuseppe Curigliano⁴, Carsten Denkert⁸, and Sherene Loi⁷

Abstract

The immune system could mediate the antitumor activity of several anticancer treatments. Several chemotherapy compounds, including anthracyclines and oxaliplatin, induce immunogenic cell death that in turn activates antitumor immune response. Trastuzumab induces antibody-dependant cell-mediated cytotoxicity. On the basis of this background, immune markers have recently been the focus of intense translational research to predict and monitor the efficacy of treatments. Gene expression arrays and immunohistochemistry have assessed immune activation and infiltration by macrophages, natural killer, and T and B lymphocytes. Using these approaches, several retrospective analyses of large trials have shown that activation of immune pathway may predict treatment efficacy and outcome in patients with breast cancers. As examples, intratumoral infiltration by lymphocytes and interferon-response in primary tumor predicted the efficacy of neoadjuvant chemotherapy. Intratumoral infiltration by lymphocytes was associated with good prognosis in patients with triple-negative breast cancer treated with adjuvant chemotherapy. More recently, it has been suggested that lymphocyte infiltration could also predict efficacy of trastuzumab. Finally, small retrospective studies have suggested that post-chemotherapy lymphocyte infiltrates could be associated with better outcome in patients who did not reach pathologic complete response. This body of evidence suggests that assessing immune infiltration and activation could be useful in the future to stratify breast cancer patients. In addition, they provide evidence for the development of immunotherapies in breast cancer patients. *Clin Cancer Res*; 19(1); 28–33. ©2012 AACR.

Background

Recent advances in the field of immunology have suggested that activation of the immune system could mediate the antitumor effects of several conventional anticancer agents, including cytotoxic, radiation therapy, antibody-based therapy, and targeted therapies. In the field of cytotoxic agents, the team of L. Zitvogel led this field by showing that anthracyclines, oxaliplatin, and radiation therapy could mediate "immunogenic" cancer cell death that will subsequently activate the immune system (1). This mechanism of immunogenic cell death was mediated by high-mobility-group box 1 (HMGB1) release and Toll-like receptor (TLR)-4 (TLR4) (1). When released after cell death induced by chemotherapy,

HMGB1 induces activation of antigen-presenting cells that in turn induce activation of immune cells (Fig. 1A).

Regarding the efficacy of monoclonal antibodies, it has been reported for a while that trastuzumab, an anti-HER2 monoclonal antibody, mediated part of its effect through the immune system. Indeed, trastuzumab only partially works in mice that are genetically defective for the receptor of immunoglobulins (Fc-gamma receptor knockout mice; ref. 2). Interestingly, some biomarker studies corroborated these findings by showing that administration of trastuzumab induced attraction of macrophages and natural killer (NK) cells, that is, cells potentially involved in antibody-dependent cell-mediated cytotoxicity (ADCC), at the tumor site (3, 4). NK cells are involved in the destruction of target cells through nonspecific mechanisms. Several preclinical and biomarker data suggest that ADCC subsequently leads to the activation and expansion of tumor-specific cytotoxic T lymphocytes (3). These latter cells specifically recognize and kill cells that present a specific antigen (adaptive immune response; Fig. 1B).

Finally, although designed to target key oncogenic events, several targeted agents exhibit immunologic properties. As an illustration, imatinib has been shown to induce NK cell activation that contributes to tumor eradication (5). Interestingly, similar findings were recently reported with endocrine therapy. Indeed, in a population of 17 patients, treatment with aromatase inhibitors were associated with changes in immune activation assessed by gene expression arrays. This modulation was associated with improved efficacy of endocrine therapy (6).

Authors' Affiliations: ¹Department of Medical Oncology, ²Institut National de la Santé et de la Recherche Médicale Unit U981, Institut Gustave Roussy, Villejuif, France; ³Department of Oncology, Hematology and Respiratory Diseases, University Hospital, Modena; ⁴Department of Medicine, Division of Medical Oncology, Istituto Europeo di Oncologia, Milano, Italy; ⁵Department of Surgery, ⁶Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; ⁷Breast Cancer Translational Research Laboratory (BCTL) J.C. Heuson, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium; and ⁸Department of Pathology, Charité Hospital, Berlin and German Breast Group, Frankfurt, Germany

Corresponding Author: Dr. Fabrice Andre, INSERM Unit U981, Department of Medical Oncology, Institut Gustave Roussy, 114 Rue Edouard Vaillant, 94805 Villejuif, France. Phone: 331-4211-4371; Fax: 331-4211-5274; E-mail: Fabrice.ANDRE@igr.fr

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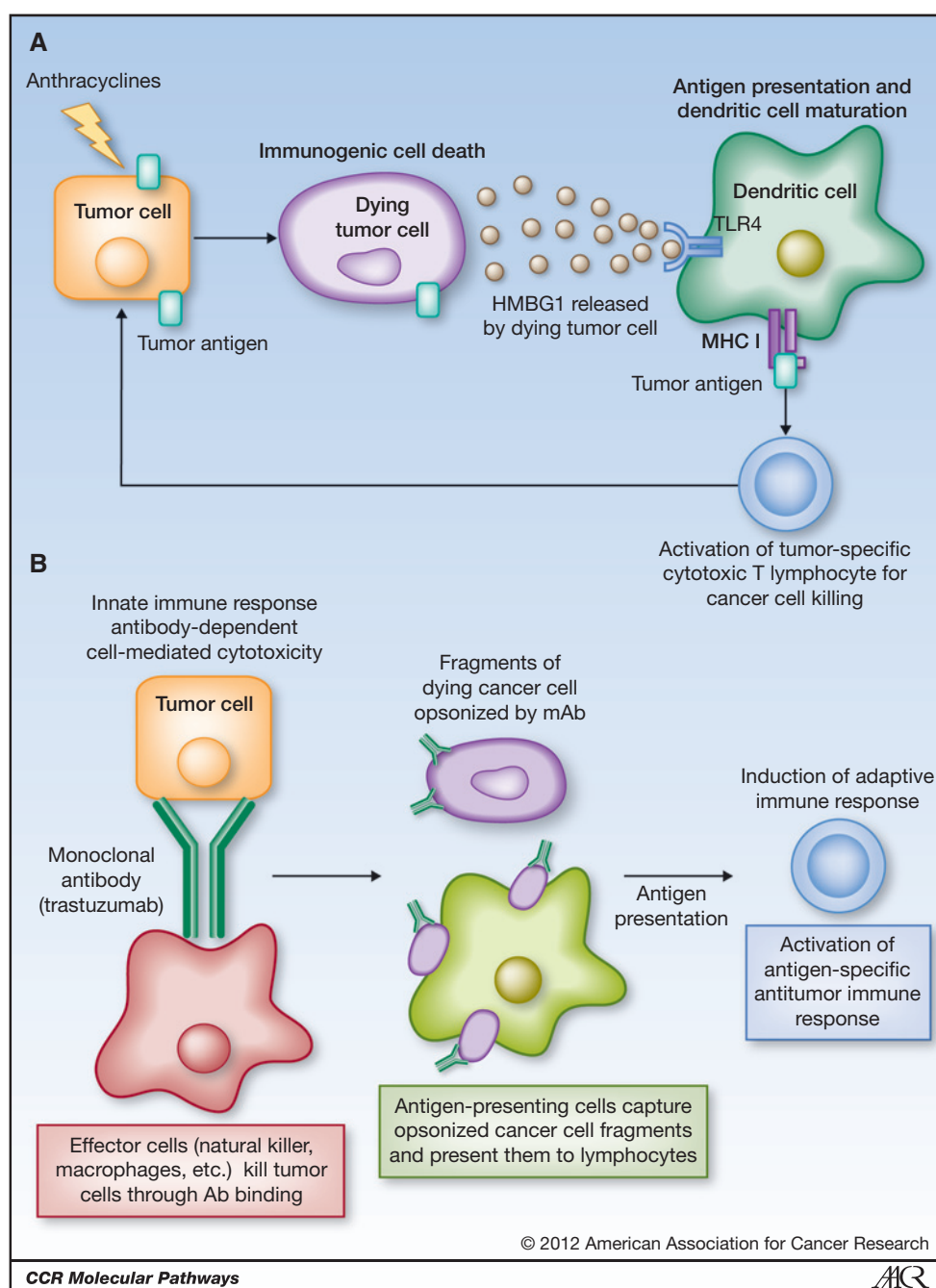


Figure 1. A, following tumor insult by cytotoxic agents, dying tumor cells release the chromatin-binding protein high-mobility group box 1 protein HMGB1 in the late phases of apoptosis. HMGB1, by interacting with TLR4 on dendritic cells, allows an optimal tumor antigen processing and presentation. Following antigen presentation, tumor-specific cytotoxic T cells are activated. B, monoclonal antibodies (mAb) bind target antigens expressed on cancer cell surface, leading to tumor cell opsonization. Recognition of tumor cells opsonized with mAbs is mediated via the Fc γ -receptor III expressed on effector cells (NK, monocytes, dendritic cells, and granulocytes). These effectors then proceed to induce tumor cell death (ADCC). The mAb-coated tumor antigens released by dying cells in the form of immune complexes are captured by dendritic cells, processed, and presented to cytotoxic and helper T cells. As a consequence, both tumor-specific cytotoxic T cells and T-helper cells are activated, the latter leading to tumor-specific B-cell stimulation and expansion and to the production of tumor-directed host antibodies.

Overall, these findings suggest that conventional therapies induce activation of the immune system, mainly tumor-specific cytotoxic T lymphocytes. These data lead to the hypothesis that (i) conventional therapies could work only in patients who present with some degree of

immune activation in the tumor site, (ii) posttreatment immune activation could identify a population of patients with good outcome, and (iii) new drugs should be developed to reverse immune defects in patients who are not able to elicit immune response after conventional

therapies. In the present article, we will address 2 questions: (i) could intratumoral immune parameters predict treatment efficacy and outcome? and (ii) what are the implications in terms of drug development?

Clinical–Translational Advances

Does intratumoral immune response predict efficacy to chemotherapy?

The most compelling data obtained in the field of immune biomarkers in breast cancer relate to the prediction of chemotherapeutic efficacy. In a recent meta-analysis of 996 patients treated with neoadjuvant chemotherapy, high immune module scores assessed by gene expression arrays were associated with an increased likelihood of pathologic complete response (pCR). Interestingly, immune module predicted pCR in all breast cancer classes [triple-negative, HER2-positive, estrogen receptor (ER)-positive/HER2-negative], and added prediction to clinical and pathologic characteristics ($P = .004$; increase in area under the curve from 0.760 to 0.836). In this study, the immune module consisted mainly in TH1/interferon response (7).

Denkert and colleagues reported similar data using another method to assess intratumor immune activation. Instead of looking at gene expression, the authors have evaluated the predictive value of the tumor-infiltrating lymphocytes (TIL). In their seminal article, they reported that TILs were independent predictors for pCR both in discovery ($n = 218$) and validation ($n = 840$) sets. TIL-positive tumors achieved between 40% and 42% of pCR, whereas the TIL-negative tumors achieved only 3% to 7% pCR. The predictive effect of TIL was mostly related to an infiltration by CD3+ (T-lymphocytes) and CD20+ (B-lymphocytes) cells (8). B lymphocytes are cells involved in antibody production after induction of immune response. This seminal article has been further validated by several studies. The same group has recently prospectively validated this finding in a population of 189 patients treated with neoadjuvant chemotherapy (9). West and colleagues also reported a predictive value for TIL infiltration in triple-negative breast cancers (TNBC; ref. 10).

Overall, consistent studies report that immune activation at baseline, assessed by pathology or gene expression array, is associated with a higher likelihood of pathologic complete response after neoadjuvant chemotherapy (3). In all reports, this parameter added value to the current clinical and pathologic characteristics.

Interestingly, chemotherapy not only induces a high rate of pCR in TIL+ patients, but can also convert a TIL– tumor into a TIL+ tumor. Symmans and colleagues have reported that taxane-based chemotherapy converted 7 of 21 breast tumors from TIL– to TIL+. Interestingly, the postchemotherapy TIL status was associated with an improved clinical response (11). Ladoire and colleagues have further explored this phenomenon. These investigators reported that neoadjuvant chemotherapy increased the CD8+ infiltration in the tumor bed. This posttreatment infiltration is associated with an improved outcome [HR, 3.85; 95% confidence interval (CI), 2.00–7.14 for patients with low CD8 infiltration; ref. 12].

Overall, these findings provide a high level of evidence that TILs at baseline is associated with high sensitivity to chemotherapy and give rise to the hypothesis that post-treatment TILs could predict outcome.

Intratumoral immune parameters to identify patients resistant to trastuzumab

Several consistent studies have suggested that trastuzumab could mediate part of its activity through the immune system. As an illustration, trastuzumab was less effective in Fc-gamma receptor knockout mice (2). On the basis of preclinical models, trastuzumab is expected to work through 2 different immune pathways. First, it mediates ADCC, that is, tumor cell killing by immune effectors. Both macrophages and NK cells have been reported to mediate ADCC (innate immune response). Innate immune response defines a cellular destruction by immune cells that is not dictated by the recognition of specific antigens. Subsequently to ADCC, and due to cell death and opsonization (complexes between cell fragments and antibodies), trastuzumab induces antigen-specific immune activation (adaptive immune response). This latter activation could generate tumor-specific cytotoxic T cells, a subset of cells that destroy target cell through recognition of foreign antigen (3). The involvement of the immune system in the trastuzumab antitumor activity was recently confirmed in mice models in two recent articles (13, 14). Overall, there is now large body of evidence from preclinical models that trastuzumab mediates its activity through the immune system. Studies indicating a possible role for the immune system in trastuzumab efficacy were initially based on samples from patients treated in the neoadjuvant setting. Arnould and colleagues have reported that trastuzumab administration was associated with an increased tumor infiltration with T lymphocytes (CD3+), macrophages (CD68+), and NK cells (NK1+; ref. 15). Gennari and colleagues reported that the level of ADCC during trastuzumab exposure was associated with a higher efficacy of trastuzumab (16). These data were confirmed in a study done in the metastatic setting (17). All of these studies suggested that trastuzumab induced immune response that in turn mediates antitumor activity. Interestingly, preliminary studies suggested that immune response was predictive not only during therapy, but also when present at baseline. Gennari and colleagues indeed reported that the lymphocyte infiltration at baseline correlated with higher trastuzumab efficacy (16). On the basis of the preliminary evidence that trastuzumab could mediate its activity through both innate and adaptive immune response, it has been hypothesized that intratumor lymphocyte infiltration could predict efficacy of trastuzumab in the adjuvant setting. Loi and colleagues have evaluated the predictive value of TIL in 935 patients randomized between chemotherapy and chemotherapy plus trastuzumab (18). TIL were assessed in a similar way as Denkert and colleagues (8). Interestingly, lymphocyte infiltration was associated with a higher trastuzumab efficacy. In patients without lymphocyte infiltration, trastuzumab was not associated with decreased risk of

relapse (HR, 1.0; 95% CI, 0.55–1.75; $P = 0.99$). In contrast, in patients with lymphocyte infiltration, trastuzumab was associated with a massive decrease in relapse risk (HR, 0.16; 95% CI, 0.031–0.81; $P = 0.013$). Interestingly, the 3-year disease-free survival (DFS) rate was 96% in patients with TIL+ tumors treated with chemotherapy and trastuzumab (18). This study confirms the potential of immune activation as a predictor for trastuzumab efficacy. Further studies will decipher which immune cell is involved in this process. Careful attention will be paid to CD68, CD11, NKp30, and CD3 infiltrations.

Overall, these pioneer studies suggest that: (i) exposure to trastuzumab leads to ADCC and tumor-specific immune activation, (ii) such immune activations correlate with higher efficacy in preliminary studies, and (iii) one retrospective study from adjuvant trials suggests that immune infiltrate predicts trastuzumab efficacy and excellent outcome.

These data lead to the perspective of stratifying patients based on lymphocyte infiltrations, and to the hypothesis that activating the immune system could sensitize to trastuzumab in high-risk patients.

Lymphocyte infiltration at baseline to predict outcome in patients optimally treated

We have previously reported that: (i) identification of high-risk patients is a major challenge for drug development in breast cancer and (ii) TILs at baseline correlate with high sensitivity to chemotherapy and trastuzumab. On the basis of these considerations, several authors have investigated whether lymphocyte infiltrations could predict outcome in patients who received adjuvant therapy. Using samples from a large randomized trial, Loi and colleagues have reported the prognostic value of TILs in patients who received anthracycline- or anthracycline-taxane-based adjuvant chemotherapy (19). The method for assessing TILs was based on Denkert and colleagues (8). Interestingly, they reported that high level of TILs was associated with a good outcome in patients with TNBC. For TNBCs with 50% or more TILs (10.5% of TNBCs), the 5-year DFS was 89% versus 62% (HR, 0.29; 95% CI, 0.11–0.81; $P = 0.018$). This parameter had no value in patients with luminal breast cancer (19). These findings were confirmed in a recent analysis of FinnHer trial (18). Finally, Liu and colleagues have reported that CD8+ lymphocyte infiltration was a strong favorable prognostic indicator in patients with basal-like breast cancer. In this latter study, HR for relapse or death was 0.35 (95% CI, 0.23–0.54) in patients with strong CD8+ cell infiltrate (20).

As reported previously, similar findings have been reported in patients with Her2-overexpressing breast cancer treated with chemotherapy and trastuzumab (18).

Overall, these findings provide evidence that TILs assessed at baseline, in addition to standard parameters, could stratify patients with TNBC or Her2-overexpressing breast cancer into high- or low-risk population. These data have been obtained using retrospective analysis of patients included in prospective large randomized trials. Several additional data from cohorts of patients treated with last-generation adjuvant regimens, together with a prospective study, are needed

before starting to implement this parameter in the context of prospective clinical trials as an inclusion criterion to select high risk TNBC or HER2-positive breast cancer.

Interestingly, in the previously mentioned studies, the TILs assessed at baseline were not helpful in identifying a population of poor prognosis luminal breast cancer. Nevertheless, this specific question requires more specific investigation in the future, especially in patients with postchemotherapy infiltrates.

Biologic nature of TIL+ TNBC and HER2-positive breast cancer

As previously shown, there is increased evidence that TIL+ TNBC and TIL+ HER2-positive breast cancer represent specific entities with good outcome and high sensitivity to chemotherapy or trastuzumab. Little is known about the molecular characteristics of TIL+ breast cancers. Two questions need to be addressed in this field. First, what is the phenotype of lymphocytes? Second, what are the genomic characteristics of TIL+ tumors?

In the pioneering study by Denkert and colleagues, TILs were reported to be both CD3+ (T lymphocytes) and CD20+ (B-lymphocytes) cells (8). Recent works have allowed better understanding about the nature of TILs. Ruffell and colleagues have reported that TILs consist mainly in CD3+/CD56– T cells, and that a minority of TILs consisted in NK cells or B lymphocytes. In this report, the vast majority of CD3+ cells were either CD4+ or CD8+ T cells. These latter cells did not express Granzyme B, suggesting that they did not present activation status at baseline. Interestingly, CD8+ cells turned to express Granzyme B after exposure to neoadjuvant chemotherapy in one-third of the patients. Finally, a minority of TILs were NK T cells (NKT), a population that combines T and NK cell characteristics (21). Overall, these data suggest that TILs are represented mainly by nonactivated T cells. Exposure to chemotherapy induced activation of these TILs in a significant proportion of cases. Unfortunately, there is no large study that investigates the clinical implications of a minority population, including NKT and NK cells. This is related to the lack of appropriate tools to assess these cells, the lack of a homogenous method for quantification, and lack of a large series. Finally, little is known about the T-cell receptor repertoire of TIL. Several studies have suggested that T-cell receptor repertoire is directed against tumor clones (22).

If we hypothesize that TILs are mediating tumor protection, one challenge in the field will be to understand which molecular mechanisms lead to lymphocyte infiltration, and how to change a TIL– tumor into a TIL+ tumor. To address these questions, there is a need to understand what the genomic characteristics of TIL+ cancers are. TILs have been correlated with CXCL9 and CXCL13 expression by the tumor. Little is known about the mechanisms that lead to chemokine release by tumors (8). Dedeurwader and colleagues have suggested that TIL+ tumors present a specific methylation pattern on immune-related genes including *CCL5* (23). Further investigations are needed to better understand whether methyltransferase and histone

deacetylases (HDAC) could regulate immune infiltration. Other mechanisms of chemokine release have been described. Andre and colleagues have shown that a cluster of chemokines is lost in a subset of breast cancers (24). Finally, it has been described that targeting TLR at the surface of cancer cells could induce chemokine release and lymphocyte attraction (25). Overall, preliminary studies suggest that regulation of chemokine release by cancer cells could be modulated. Further studies should investigate whether HDAC inhibitor, TLR-L, could change a TIL– tumor into a TIL+ cancer.

A specific case: the tumor infiltration by regulatory T cells

Regulatory T cells (Treg) mediate tumor suppression and tumor growth. These cells are characterized by expression of CD4, CD25, and FoxP3. Several works have reported that Treg could be associated with worse prognosis in patients with early breast cancer (26, 27). Interestingly, cytotoxic agents could modulate Treg infiltrations. As an illustration, Ladoire and colleagues have shown that chemotherapy decreases FoxP3 infiltration and that such decrease correlates with good outcome (28).

Involvement of immune system in treatment response for other cancer types

As previously mentioned, the immune system plays a major role in treatment efficacy. Although the article focuses on breast cancer, evidence of the involvement of the immune system in drug efficacy has been reported in other tumor types. Liu and colleagues (29) have suggested that the level of CD8/foxP3 infiltration could predict the efficacy of neoadjuvant cisplatin-based chemotherapy. Similar findings were reported in colorectal cancer in which Halama and colleagues (30) have shown that TILs predicted sensitivity to chemotherapy in the metastatic setting. Finally, in the field of targeted agents, Imatinib has been reported to mediate part of its activity via the activation of NK cells (5).

Conclusion: What are the implications in terms of drug development?

These new findings could have two major impacts in drug development for breast cancer. First, if the prognostic value of TILs is confirmed in TNBC and HER2-positive breast cancers, one could suggest that future prospective clinical trials stratify outcome data by these factors.

The second implication of such findings relates to the development of immune strategies in breast cancer. Three strategies could be developed. First, considering results obtained in other tumor types, there is a strong rationale to develop anti–programmed death 1 (PD1) or anti–programmed death 1 ligand (PDL1) antibodies in breast cancers (31, 32). PD1 is a T-cell receptor that inhibits antitumor immune response. By administering anti-PD1 (or anti-PDL1) antibodies, it is expected to activate the immune system, previously inhibited by tumor cell. Preclinical studies have already shown a major synergism between PD1 antibody and trastuzumab in preclinical models (13). In addition to PDL1,

such studies should stratify breast cancer patients based on TILs. Indeed, there is a need to better understand whether drugs targeting immune checkpoints activate *in situ* lymphocytes or induce lymphocyte attraction in the tumor bed. In this regard, the preoperative breast cancer model is very appropriate and could add knowledge to the field. The second strategy could consist of changing a TIL– tumor into a TIL+ tumor. Ligands for TLR have been shown to mediate chemokine release by cancer cells and lymphocyte attraction. Several compounds are being investigated (33). Finally, tumor vaccines could be developed as in other cancer types (34).

Overall, there is increasing evidence that TIL+ breast cancers present specific features that could have specific clinical implications. First, a large body of evidence from population-based studies or randomized trials suggests that TIL+ TNBC and TIL+ HER2-positive breast cancer present a good prognosis. These robust data have been obtained in independent cohorts from independent teams. They will need additional validation using a prospective trial before implementation. Interestingly, a study has evaluated the prognostic role of gene expression modules related to key biologic processes in breast cancer. In this analysis, the immune response-related signature was significantly associated with a worse clinical outcome in both the HER2-positive and ER-negative/HER2-negative subtypes (35). The first implication could be to use TILs as inclusion criteria in large adjuvant trials that include TNBC or HER2-positive breast cancer. The second prognostic implication relates to the potentially good prognosis of patients who were TIL– at baseline, but became TIL+ after neoadjuvant therapy. These data have been obtained in several small retrospective studies that can only be considered hypothesis generating. Nevertheless, if these data are confirmed, they could be used to better stratify which patients should be included in postneoadjuvant trials. Finally, the information that TIL positivity is associated with good outcome is an argument to develop immune strategies in breast cancers. The most robust data in the preclinical setting suggest combining anti-PD1 and anti-HER2 antibodies. The testing of these trials should include both patients with TIL+/high tumor burden and TIL– tumors.

Disclosure of Potential Conflicts of Interest

F. Andre has an ownership interest (including patents) and is an inventor of a patent related to TLR3 targeting in cancer under development by Innate Pharma. C. Sotiriou has an ownership interest (including patents) and is a co-owner of patents related to gene expression prognostic signatures. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: F. Andre, S. Loi

Development of methodology: S. Loi

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. Loi

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Denkert, S. Loi

Writing, review, and/or revision of the manuscript: F. Andre, M.V. Dieci, P. Dubsy, G. Curigliano, C. Denkert, S. Loi

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Andre

Study supervision: F. Andre

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