Prognostic Relevance of Nuclear Receptors in Relation to Peritumoral Inflammation and Tumor Infiltration by Lymphocytes in Breast Cancer

Melitta B. Köpke¹, Marie-Christine Chateau², Florence Boissière-Michot², Mariella Schneider¹, Fabian Garrido¹, Alaleh Zati-Zehni³, Theresa Vilsmaier³, Mirjana Kessler³, Nina Ditsch¹, Vincent Cavaillès⁴,*,† and Udo Jeschke¹,*,†

¹ Department of Obstetrics & Gynecology, University Hospital Augsburg, 86156 Augsburg, Germany
² Translational Research Unit, Montpellier Cancer Institute Val d’Aurelle, 208 rue des Apothicaires, F-34298 Montpellier, France
³ Department of Obstetrics and Gynecology, University Hospital, LMU Munich, 81377 Munich, Germany
⁴ IRCM-Institut de Recherche en Cancérologie de Montpellier, INSERM U1194, Université Montpellier, Parc Euromédecine, 208 rue des Apothicaires, F-34298 Montpellier, France
* Correspondence: vincent.cavailles@inserm.fr (V.C.); udo.jeschke@med.uni-augsburg.de (U.J.);
Tel.: +33-4-11-28-31-72 (V.C.); +49-821-400-165505 (U.J.)
† These authors contributed equally to this work.

Simple Summary: The aim of this study was to investigate the prognostic impact of tumor-infiltrating lymphocytes (TILs) in a panel of 264 sporadic breast cancers by quantifying TIL levels according to Salgado and correlate this with type I and II nuclear receptor expression. Breast cancer cases with a TIL Salgado score of >15% showed a significantly decreased overall survival and peritumoral inflammation (according to Klintrup) determined the prognostic value of ER, PR, and PPARγ in BC. Therefore, the present study demonstrates significant relations between TIL levels, nuclear receptor expression and prognosis in breast cancer.

Abstract: The prognostic impact of tumor-infiltrating lymphocytes (TILs) is intensively investigated in breast cancer (BC). It is already known that triple-negative breast cancer (TNBC), the most aggressive type of BC, has the highest percentage of TILs. In addition, there is an influence of steroid hormone receptor expression (type I nuclear receptors) on TIL subpopulations in breast cancer tissue. The link between type II nuclear receptors and the level of TILs is unclear. Therefore, the aim of this study was to quantify TILs in a panel of 264 sporadic breast cancers and investigate the correlation of TIL levels with type I and II nuclear receptors expression. TIL levels were significantly increased in the subgroup of TNBC. By contrast, they decreased in estrogen (ER)- or progesterone receptor (PR)-positive cases. Moreover, TIL levels were correlated with type II nuclear receptors, including PPARγ, with a significant inverse correlation of the nuclear form (r = −0.727, p < 0.001) and a weak positive correlation of the cytoplasmic form (r = 0.202, p < 0.002). Surprisingly, BC cases with a TIL Salgado score of >15% showed a significantly decreased overall survival. In addition, peritumoral inflammation was also quantified in BC tissue samples. In our cohort, although the level of peritumoral inflammation was not correlated with OS, it determined the prognostic value of ER, PR, and PPARγ in BC. Altogether, the present study provides a differentiated overview of the relations between nuclear receptor expression, TIL levels, peritumoral inflammation, and prognosis in BC.

Keywords: tumor-infiltrating lymphocytes (TILs); peritumoral inflammation; nuclear receptors; prognosis; breast cancer
1. Introduction

Although the prognostic impact of tumor-infiltrating lymphocyte (TIL) populations in breast cancer (BC) is still debated, triple-negative breast cancer (TNBC) clearly shows a higher density of TILs as compared with other BC subtypes [1–4]. This is probably due to their higher tumor mutational burden leading to an increased number of antigenic tumor variants and neoepitope load [5]. TILs comprise different populations of lymphocyte subtypes (T and B cells) and natural killer (NK) cells. Furthermore, macrophages and dendritic cells (DCs) are also present in the tumor environment [6–12]. Originally, peritumoral inflammation was evaluated by a quantification score developed by Klintrup et al. [13] for colorectal cancer, and the inflammatory reaction was divided into four categories. Later on, the morphological evaluation of TILs was assessed by examination of hematoxylin- and eosin-stained tumor sections and standardized by an international group of pathologists, published by Salgado et al. [14] and generally known as the Salgado score [15].

As already stated, TNBCs show a higher density of TILs than other BC subtypes, and estrogen (ER) and progesterone receptor (PR) (type I nuclear receptors) correlation with TIL density has already been described by a number of studies [16–18]. In TNBC, on the other hand, stromal TILs are considered a strong prognostic factor, and patients with a high TIL density show better survival [19]. Although the links between TILs and steroid hormone receptors (type I nuclear receptors) are intensely studied in BC [20], the association between type II nuclear receptors and TILs has not been investigated. Type II nuclear receptors form heterodimers with RXR and consist of a variety of subtypes, including thyroid hormone and vitamin D receptors, PPARs, AhR, LXR, and others [21–28]. Their role in BC biology and their impact on patient survival have been reported by our group and others [24,29–33].

Because studies on the link between type II nuclear receptors, TILs, and inflammatory cell reaction in BC are lacking, the aim of this work was to investigate correlations between TILs or peritumoral inflammation and type I and II nuclear receptors and their influence on patient survival.

2. Materials and Methods

2.1. Materials

This study is based on the use of a cohort consisting of 264 formalin-fixed paraffin-embedded primary BC tissues (see Supplementary Table S1) that were collected from patients who underwent surgery between 2000 and 2002 at the Department of Gynecology and Obstetrics of the Ludwig-Maximilian-University in Munich, Germany (clinicopathological characteristics of the patients are provided in the supplementary data). After an observation period of more than 10 years, disease-free survival (DFS) and overall survival (OS) were statistically analyzed. The follow-up data for this cohort were retrieved from the Munich Cancer Registry. The tissue samples used in this study were leftover material after all diagnostics had been completed and were retrieved from the archive of Gynecology and Obstetrics, Ludwig-Maximilian-University, Munich, Germany.

2.2. Ethical Approval

All patient data and clinical information from the Munich Cancer Registry were fully anonymized and encoded for statistical analysis. The study was performed according to the standards set in the Declaration of Helsinki 1975. The current study was approved by the ethics committee of the Ludwig-Maximilian-University Munich, Germany (approval number 048–08). The authors were blinded from the clinical information during experimental analysis.

2.3. Expression of Nuclear Receptors

Using the above-described BC cohort, the expression of type I and type II nuclear receptors has been previously analyzed by immunohistochemistry by our group. Information was specifically evaluated regarding ERα and PR [34], PPARγ [35], thyroid hormone receptors (TRs) [36,37], AhR [33], LXR [38], and RXRα [39,40].
2.4. TIL Quantification

Tumor-infiltrating lymphocytes (TILs) were quantified by an experienced gynaecopathologist (M-C.C). Figure 1 shows representative pictures of low and high expression of the evaluated nuclear receptors. Scoring was based on the method developed by Salgado et al. specifically for the evaluation of TILs in BC tissue [14]. According to this method, stromal TILs within the tumor are scored as a percentage of the stromal areas alone (areas occupied by carcinoma cells are not included in the assessed area).

2.3. Expression of Nuclear Receptors

Using the above-described BC cohort, the expression of type I and type II nuclear receptors has been previously analyzed by immunohistochemistry by our group. Information was specifically evaluated regarding ERα and PR [34], PPARγ [35], thyroid hormone receptors (TRs) [36,37], AhR [33], LXR [38], and RXRα [39,40].

2.4. TIL Quantification

Tumor-infiltrating lymphocytes (TILs) were quantified by an experienced gynaecopathologist (M-C.C). Figure 1 shows representative pictures of low and high expression of the evaluated nuclear receptors. Scoring was based on the method developed by Salgado et al. specifically for the evaluation of TILs in BC tissue [14]. According to this method, stromal TILs within the tumor are scored as a percentage of the stromal areas alone (areas occupied by carcinoma cells are not included in the assessed area).

We also adapted the scoring method of Klintrup et al. [13], developed originally for the quantification of inflammatory cell reaction in colorectal cancer at the invasive margin, therefore representing immune cells around the tumor, and classified into four categories:

- score 0 = no inflammatory cells at the invasive margin;
- score 1 = mild and patchy increase of inflammatory cells at the invasive margin;
- score 2 = increased inflammatory cells forming a band-like infiltration at the invasive margin;
- score 3 = prominent inflammatory reaction forming a cup-like zone at the invasive margin.

Figure 1. Representative microphotographs of low and high expression of ERα (estrogen receptor alpha), PR (progesterone receptor), and PPARγ (peroxisome proliferator-activated receptor gamma) in 25× magnification.

2.5. Statistical Evaluation

Statistical analysis was performed with the IBM Statistical Package for the Social Sciences (IBM SPSS Statistic v26.0 Inc., Chicago, IL, USA). The gathered results were inserted into the SPSS database in the implied manner. Correlations between findings of immunohistochemical staining were performed with Spearman’s analysis. The nonparametric
Kruskal–Wallis for more than two independent groups or the Mann–Whitney U test was used to test for differences in TIL density regarding the set prognostic markers. OS (in years) and DFS (in years) were compared by Kaplan–Meier graphics, and differences in patient survival times were tested for significance using the chi-square statistics of the log-rank test. For multivariate analyses, the Cox regression model for survival was used, and the following factors were included: age of the patient, pT and pN of the TNM staging system, grading, and histology type. Each parameter considered significant showed a value of \( p < 0.05 \). The \( p \)-value and the number of patients analyzed in each group are given for each chart.

3. Results

3.1. Quantification of TILs in the Tumor Stroma

Quantification of TILs according to the Salgado score revealed that the majority of the tumors exhibited no more than 10% TILs, with 183 cases (69.3%) showing up to 10% TILs, whereas only 26 cases (9.9%) showed more than 40% TILs (exact distribution is presented in Table 1).

Table 1. TIL quantification according to the Salgado score.

| % TILs | Frequency | Percent | Cumulative Percent of Accessible Cases |
|--------|-----------|---------|---------------------------------------|
| 1      | 32        | 12.1    | 12.5                                  |
| 5      | 56        | 21.3    | 34.5                                  |
| 10     | 95        | 35.9    | 71.8                                  |
| 20     | 27        | 10.2    | 82.4                                  |
| 30     | 19        | 7.2     | 89.8                                  |
| 40     | 8         | 3.0     | 92.9                                  |
| 50     | 4         | 1.5     | 94.5                                  |
| 60     | 5         | 1.9     | 96.5                                  |
| 70     | 7         | 2.7     | 99.2                                  |
| 80     | 1         | 0.4     | 99.6                                  |
| 90     | 1         | 0.4     | 100                                   |
| Not assessable | 9 | 3.4 | |
| Total  | 264       | 100     | |

3.2. TIL Density According to BC Subtypes

As expected, TNBC cases showed the highest TIL counts (13.33% of all cases), as evaluated by the Salgado score (median 26.6%; triple negative and 6.0%; remaining cases; \( p < 0.001 \); Figure 2A). In addition, TIL density was significantly elevated (\( p = 0.008 \)) from G1 to G3 carcinomas (Figure 2B). Grading was performed according to the Elston and Ellis criteria [41]. Comparing the BC molecular subtypes, we found significantly higher TIL levels in basal-like and in the two Her2 subtypes (luminal and nonluminal) in comparison with luminal A and luminal B molecular subtypes (Figure 3). Supplementary Table S1 contains patient numbers for all groups.
3.3. Prognostic Relevance of TIL Levels

Kaplan–Meier curve visualized a significant negative association of the OS (Figure 4) when TIL levels were higher than 15%, as assessed by the Salgado score in the whole BC population. A statistically negative significant correlation was observed for the OS ($p = 0.02$), calculated by the log-rank test.
Figure 4. Kaplan–Meier survival analyses of TIL density, according to the Salgado score, revealed significant differences in OS. Patients with TIL levels greater than 15% showed significantly reduced OS (mean 8.6 years) compared with patients with lower TIL levels (mean OS 10.4 years; \( p = 0.020 \)).

However, as shown in Table 2, multivariate Cox regression did not identify the level of TILs as an independent prognostic factor for OS (HR 1.967, 95%CI 0.921–4.200, \( p = 0.081 \)). Only age at surgery was significant.

Table 2. Multivariate Cox regression analysis of TIL levels (Salgado score) regarding OS.

|                          | Significance | Hazard Ratio | 95% CI      |
|--------------------------|--------------|--------------|-------------|
|                          |              |              | Lower      | Upper      |
| TIL level (>15%)         | 0.081        | 1.967        | 0.921      | 4.200      |
| Age at surgery           | 0.005        | 1.037        | 1.011      | 1.064      |
| pT                       | 0.153        | 1.193        | 0.936      | 1.520      |
| pN                       | 0.220        | 1.167        | 0.912      | 1.492      |
| Stage                    | 0.527        | 0.694        | 0.224      | 2.153      |
| Grading                  | 0.107        | 1.683        | 0.893      | 3.171      |
| Histology                | 0.460        | 1.010        | 0.984      | 1.036      |

3.4. Correlation of TILs with Nuclear Receptor Expression

As expected, BC cases with ER expression (80% of all cases) showed a significantly lower level of TILs as evaluated by the Salgado score (median 9.3%; ER\(\alpha\) positive and 23.3%; ER\(\alpha\) negative; \( p < 0.001 \); Figure 5A). A similar result was obtained by analyzing PR expression; PR-positive BC (57% of all cases) also showed a significantly lower expression of TILs as evaluated by the Salgado score (median 15.7%; PR positive and 16.9%; PR negative; \( p = 0.003 \); Figure 5B).
Figure 5. Level of TILs evaluated by the Salgado score is significantly higher in cases with no expression of ERα (compared with cases with ERα expression (A); \( p < 0.001 \)). Level of TILs is also significantly higher in cases with no PR expression (B); \( p = 0.003 \). The numbers represent outliers, and the circles represent outlier cases. Extreme outliers are marked with an (★) on the boxplot.

Significant correlations were also observed with type II nuclear receptors. Indeed, the nuclear forms of PPARγ and LXR showed a negative correlation with TIL density. By contrast, the level of TRβ and RXRα expressed in the nucleus showed a very weak positive correlation with TIL density. In addition to the nuclear expression, type II nuclear receptors were also detected in the cytoplasm. We identified weak but significant positive correlations between TILs and cytoplasmic type II receptors (Table 3).

Table 3. Correlation of nuclear and cytoplasmic staining of type II nuclear receptors with TILs assessed by the Salgado score (green or orange: weak negative or positive correlation \( r < 0.39 \); red: strong negative correlation \( r > 0.60 \)).

| Nuclear Receptor Parameter | Value |
|---------------------------|-------|
| Nuclear PPARγ Correlation Coefficient | –0.727 |
| Sig. (2-tailed) | <0.001 |
| N | 237 |
| Cytoplasmic PPARγ Correlation Coefficient | 0.202 |
| Sig. (2-tailed) | 0.002 |
| N | 237 |
| Nuclear LXR Correlation Coefficient | –0.254 |
| Sig. (2-tailed) | <0.001 |
| N | 250 |
| Cytoplasmic TRα Correlation Coefficient | 0.197 |
| Sig. (2-tailed) | 0.002 |
| N | 240 |
| Cytoplasmic TRα1 Correlation Coefficient | 0.203 |
| Sig. (2-tailed) | 0.002 |
| N | 237 |
| Nuclear TRβ Correlation Coefficient | 0.172 |
| Sig. (2-tailed) | 0.007 |
| N | 242 |
Table 3. Cont.

| Nuclear Receptor  | Parameter         | Value     |
|-------------------|-------------------|-----------|
| Cytoplasmic TRβ   | Correlation Coefficient | 0.279     |
|                   | Sig. (2-tailed)   | <0.001    |
|                   | N                 | 242       |
| Nuclear RXRα      | Correlation Coefficient | 0.137     |
|                   | Sig. (2-tailed)   | 0.029     |
|                   | N                 | 255       |

3.5. Quantification of Peritumoral Inflammation

Quantification of peritumoral inflammation adapted from the Klintrup score revealed 74 cases (30%) with no inflammatory cells at the invasive margin, 143 cases (57.9%) with mild and patchy increase in inflammatory cells, and 30 cases (12.1%) with increased inflammatory cells forming a band-like infiltration at the invasive margin (Table 4). None of the 247 assessable samples showed a prominent inflammatory reaction at the invasive margin (score 3, according to Klintrup criteria). A total of 17 cases were not assessable. Examples of tumors with different TIL densities according to the Klintrup score is shown in Figure 6.

Table 4. Quantification of peritumoral inflammation according to the Klintrup score.

| Peritumoral Inflammation                                           | Frequency | Percent | Cumulative Percent of Accessible Cases |
|-------------------------------------------------------------------|-----------|---------|---------------------------------------|
| Score 0—no inflammatory cells at the invasive margin              | 74        | 28.0    | 30                                    |
| Score 1—mild and patchy increase of inflammatory cells at the invasive margin | 143       | 54.2    | 87.9                                  |
| Score 2—increased inflammatory cells forming a band-like infiltration at the invasive margin | 30        | 11.4    | 100                                   |
| Not assessable                                                   | 17        | 6.4     |                                       |
| Total                                                             | 264       | 100     |                                       |

As shown in Figure 7, quantification of peritumoral inflammation on BC tissue samples according to the Klintrup score was not associated with OS differences.
3.6. Correlation of Peritumoral Inflammation with Nuclear Receptor Expression

Concerning nuclear receptor expression and peritumoral infiltration, we found significantly more ER- and PR-positive cases in patients with no or mild and patchy increase in inflammatory cells at the invasive margin compared with cases with increased inflammatory cells forming a band-like infiltration at the invasive margin ($p < 0.001$ and $0.017$, respectively).

Analyses of type II receptors revealed a significant increase in cytoplasmic PPARγ in cases with no or mild and patchy increase in inflammatory cells compared with cases with increased inflammatory cells (Figure 8A, $p = 0.002$). By contrast, expression of PPARγ in the nucleus was significantly reduced in cases with no or mild and patchy increase in inflammatory cells compared with cases with increased inflammatory cells (Figure 8B, $p = 0.003$). Analyses of other type II receptors also revealed a significant correlation between cytoplasmic THRβ or nuclear LXR and peritumoral inflammation (Suppl. Figures, $p = 0.045$ and 0.026, respectively).

Type-specific analyses (luminal A, luminal B, basal-like, HER2-positive) were performed to correlate peritumoral inflammation with type II nuclear receptors but did not yield significant results due to the small number of cases (data not shown).
3.7. Prognostic Relevance of Nuclear Receptors Expression according to Peritumoral Inflammation

Although peritumoral inflammation had no prognostic relevance in our BC cohort, we asked whether it might influence the prognostic relevance of nuclear receptors, including ERα and PR. ERα has been known for decades as a positive prognostic marker for BC survival. We analyzed OS of ERα-positive and -negative patients according to peritumoral inflammation. Kaplan–Meier survival analyses visualized a significant positive correlation of ERα expression only in the subgroups with no (score 0) inflammatory cells at the invasive margin. Patients being ERα-negative showed a mean survival time of 4.2 years compared with 10.3 years for ERα-positive patients (Figure 9A, \( p < 0.001 \)). There is also a significant difference in OS between ERα-positive and -negative patients in the tumor with score 1, i.e., those with mild and patchy increase in inflammatory cells at the invasive margin. Patients being ERα-negative showed a mean survival time of 8.0 years compared with 10.6 years for ERα-positive patients (Figure 9B, \( p = 0.013 \)). By contrast, in patients with an increased inflammatory pattern at the invasive margin (score 2), the lack of ERα expression was no longer a marker of poor prognosis (Figure 9C, \( p = 0.642 \)).

**Figure 8.** Expression of PPARγ varies according to peritumoral inflammation. Cytoplasmic PPARγ is significantly higher in cases with peritumoral immune infiltration (A), whereas an inverse correlation is observed with the levels of nuclear PPARγ (B). Mild outliers are marked with a circle (○) on the boxplot. Extreme outliers are marked with an (★) on the boxplot.

**Figure 9.** Kaplan–Meier survival analyses for estrogen receptor (ERα) according to the level of peritumoral inflammation with no inflammatory cells at the invasive margin (Klintrup Score 0 (A)), mild and patchy increase of inflammatory cells (Klintrup Score 1(B)) and increased inflammatory cells (Klintrup Score 2 (C)) on overall survival (OS).
Progestosterone receptor is also a positive prognostic marker for BC survival. We analyzed OS of PR-positive and -negative patients according to peritumoral inflammation. Kaplan–Meier survival analyses visualized a significant positive association with long OS if PR is positive only in the subgroup with no inflammatory cells at the invasive margin (Figure 10A). Patients being PR-positive showed a mean survival time of 10.6 years compared with 7.6 years for PR-negative patients ($p = 0.033$). By contrast, there was no significant difference in OS between PR-positive and -negative patients having tumors with mild or increased peritumor inflammation (Figure 10B,C, $p = 0.070$ and $p = 0.319$, respectively).

![Figure 10](image1.png)

**Figure 10.** Kaplan–Meier survival analyses for progesterone receptor (PR) in relation to the level of peritumoral inflammation with no inflammatory cells at the invasive margin (Klintrup Score 0 (A)), mild and pathy increase of inflammatory cells (Klintrup Score 1(B)) and increased inflammatory cells (Klintrup Score 2 (C)) on overall survival (OS).

In contrast to type I nuclear receptors, cytoplasmic PPARγ type II nuclear receptor was recently reported by our group to be a negative prognostic marker for breast cancer survival [35]. We analyzed the overall survival of cytoplasmic PPARγ-positive and -negative patients in the three above-described subgroups (score 0–2 of peritumoral inflammation). Kaplan–Meier survival analysis visualized a significant negative association of cytoplasmic PPARγ with OS only in the subgroup with no inflammatory cells at the invasive margin (Figure 11A). Patients with tumors being negative for cytoplasmic PPARγ showed a mean survival time of 10.7 years compared with 7.7 years for patients with tumors positive for cytoplasmic PPARγ ($p = 0.008$). No significant difference in OS was observed between cytoplasmic PPARγ-positive and -negative patients in BC with mild or increased peritumoral inflammation (Figure 11B,C, $p = 0.280$ and C, $p = 0.225$, respectively). As shown in Supplementary Figures S1–S3, no significant differences were observed concerning the correlation of RXRα, LXR, and AhR expression with patient survival in relation to the levels of peritumoral inflammation. Finally, when the same type of analyses as the ones shown in Figures 9–11 was performed using a classification based on the Salgado score, we observed no influence on the correlation between ERα, PR, or PPARγ expression and survival (data not shown). This could be explained by the fact that the two quantification scores exhibited differences in tumor classification, as shown in Supplementary Table S2. Although all tumors with high peritumoral inflammation had a TIL density of >30%, the groups with no or mild peritumoral inflammation were more heterogeneous in terms of TIL levels.
TIL density and type II nuclear receptor expression was not investigated before. TIL density only if expressed in the cytoplasm. Nuclear expression of PPARs and their subcellular localization play notable roles in the pathophysiology of BC as well [34,42]. As the subcellular localization of nuclear receptors, e.g., RXRα or PPARγ, have such an impact on prognosis, one can assume that they exert specific functions in the cytoplasm, as has been proposed for PPARs [43].

In concordance with already published results, we found significantly higher rates of TILs in triple-negative breast cancer (TNBC). As known from former studies, TNBCs showed a higher density of TILs than other BC subtypes, probably because of their higher number of antigenic tumor variants, neoepitope load, and tumor mutational burden [5]. In TNBC, stromal TILs are considered a strong prognostic factor, and patients with a high TIL density show better survival [1–3,44–49]. However, in our panel, no effect of TILs on clinical outcome was observed in the group of TNBC, probably because the number of samples was too small (34 cases) to reach significance. In addition, we confirmed that TIL levels are significantly elevated in Her2-positive and basal-like breast cancer [50]. Luminal types of BC showed the significantly lowest level of TILs in our study group.

As expected, in our BC cohort, ERα- or PR-negative cases showed higher TIL density. Interestingly, cases with high cytoplasmic PPARy expression also showed significantly elevated TIL levels. In a recent study on the same patient cohort, we analyzed the combined cytoplasmic expression of RXRα and PPARγ. Patients with tumors expressing both NRs in the cytoplasm of tumor cells exhibited significantly shorter OS and DFS [39]. Based on those results, we investigated the correlation between TIL levels and the expression of nuclear type II receptors. The main member of this group is RXRα because all other members of this subfamily form heterodimers with RXRα. Interestingly, nuclear RXRα showed a positive correlation with TIL density as only the thyroid hormone receptor TRβ did, whether the latter was expressed in the nucleus or the cytoplasm of tumor cells. All other type II receptors (PPARγ, TRα, TRα1, and TRβ) showed a positive correlation with TIL density only if expressed in the cytoplasm. Nuclear expression of PPARγ and LXR resulted in a negative correlation with TIL levels. To our knowledge, the concordance of TIL density and type II nuclear receptor expression was not investigated before.

Concerning the correlation between TILs and survival, we could show that TILs (using the Salgado score and 15% TILs as cutoff value) have poor prognostic value in OS in our
BC cohort. This result might be explained by the fact that the studied cohort is highly heterogeneous in BC subtypes, ER, and HER2 status or histology (ductal, lobular, and others). In this cohort, and as expected, the basal samples (a subpopulation that includes TNBC in a vast majority) are those displaying the highest TIL density. It is well known that these tumors are also those with the worst clinical outcome. Indeed, whereas it is well known that TILs are associated with better clinical outcomes in TNBC- and HER-2 positive breast cancer [51,52], the study of Desmedt, Salgado et al. described that TIL levels were associated with worse prognostic outcomes in lobular carcinoma [50]. Thus, the association of poor clinical outcomes with high TILs density could reflect sample heterogeneity.

We then analyzed the influence of the peritumoral infiltrate on the prognostic value of nuclear receptors. Although the prognostic value of TIL levels in the ER-negative/Her2-negative breast cancer population is well known, the impact of immune infiltration on the prognosis value of NRs was never investigated before [53–55]. ERα (and steroid hormone receptor expression in general) is known to be favorable concerning OS [56–58] in BC. Very interestingly, our study clearly showed that the positive correlation of steroid hormone receptor expression with prognosis is dependent on the level of peritumoral inflammation. Indeed, ERα exhibited a prognosis value only for patients with tumors having low levels of inflammation at the invasive margin. A similar result was obtained with the progesterone receptor (PR): only the subgroup with no peritumoral inflammation showed significant differences in overall survival based on PR positivity. In concordance with the results obtained with the steroid hormone receptor, the cytoplasmic expression of PPARγ was found to be a negative prognosticator only in the group without peritumoral inflammation.

Concerning the impact of the immune peritumoral infiltrate on the prognosis value of ERα, the results shown in Figure 9 confirmed that some ER-negative tumors are not immunogenic (at least do not present an inflammatory cell reaction) and, consequently, are more aggressive and lead to a short OS of patients. The molecular mechanisms of this observation remain to be deciphered but could be related to Ras/MAPK pathway activation in these TNBC samples. Indeed, it has been reported that this pathway may promote host antitumor immune evasion in tumor cell-autonomous pathways [59]. A striking result was the link between cytoplasmic expression of PPARγ and peri- or intratumor immune infiltration. Cytoplasmic expression of PPARγ was accompanied with an increase in immune infiltration. It is already known that the expression of PPARγ, as the key regulator of lipogenesis, is altered in breast cancer [60]. We could show very recently that cytoplasmic PPARγ is a negative prognosticator in breast cancer [35,39]. PPARγ can determine the cellular phenotype by regulating differentiation and function by activating the transcription of PPARγ target genes [61]. Similar molecular mechanisms are in place in immune cells, and also, here, PPARγ can determine cellular phenotype [61].

This study has some limitations, considering its retrospective nature, the relative heterogeneity of the cohort, and the way TILs was assessed. For instance, it might have been interesting to analyze the influence of peritumoral infiltrate on the prognostic value of nuclear receptors within different molecular subgroups of BC, in particular in the TNBC. In addition, we herein only performed a global analysis of TILs, and since these cells may be immunogenic or immune-suppressive, more precise methods based on immunohistochemical detection of the different lymphocyte subtypes (including cytotoxic and regulatory T cells, or B/plasma cells) would have been more informative. These points will be addressed in further studies, which are also needed to determine whether the prognosis value of cytoplasmic PPARγ, combined with a lack of peritumoral infiltration, could become important in clinical routine and influence therapy decisions.

5. Conclusions

Altogether, this study is one of the first that describes the correlation between the expression of nuclear receptors (in particular PPARγ) and peritumoral inflammation or TIL levels in relation to prognosis in BC. Although some studies exist on PPARα in melanoma [62–64], PPARγ has, to our knowledge, never been investigated in relation
to TILs and survival in any form of cancer. In our whole cohort of sporadic breast cancers, PPARγ expression in the cytoplasm of cancer cells was positively correlated with TIL levels and peritumoral inflammation. In addition, the prognostic value of cytoplasmic PPARγ was determined by the level of immune peritumoral infiltrate.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14194561/s1, Figure S1: Kaplan-Meier survival analyses for cytoplasmic RXRα in relation to the level of peritumoral inflammation on overall survival (OS). Figure S2: Kaplan-Meier survival analyses for cytoplasmic LXR in relation to the level of peritumoral inflammation on overall survival (OS). Figure S3: Kaplan-Meier survival analyses for cytoplasmic AHR in relation to the level of peritumoral inflammation on overall survival (OS). Figure S4: Expression of LXR in the nucleus is significantly decreased from cases with no peritumoral infiltration of immune cells to cases with increased numbers of infiltrating immune cells. In addition, expression of cytoplasmic expressed thyroid hormone receptor beta increases from cases with no immune cell infiltration to cases with increased infiltration of inflammatory cells. Table S1: Clinical and pathological characteristics of all patients. Table S2: Correlation between Salgado and Klintrup Score.

Author Contributions: Conceptualization, V.C. and U.J.; methodology, N.D. and M.K.; software, F.G.; validation, M.S., A.Z.-Z., and T.V.; formal analysis, M.B.K.; investigation, M.-C.C.; resources, F.B.-M.; data curation, F.B.-M.; writing—original draft preparation, M.B.K. and U.J.; writing—review and editing, V.C.; visualization, N.D.; supervision, U.J.; project administration, V.C.; funding acquisition, N.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the LMU Munich (approval number 048-08; 18 March 2008).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data supporting the reported results can be obtained from the corresponding author.

Acknowledgments: The authors thank Christina Kuhn for the immunohistochemical data.

Conflicts of Interest: N.D. reports funding from MSD, Novartis, Pfizer, Roche, AstraZeneca, TEVA, Mentor, and MCI Healthcare. All other authors report no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References
1. Adams, S.; Gray, R.J.; Demaria, S.; Goldstein, L.; Perez, E.A.; Shulman, L.N.; Martino, S.; Wang, M.; Jones, V.E.; Saphner, T.J.; et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase iii randomized adjuvant breast cancer trials: Ecog 2197 and ecog 1199. J. Clin. Oncol. 2014, 32, 2959–2966. [CrossRef] [PubMed]
2. Dieci, M.V.; Criscitiello, C.; Goubar, A.; Viale, G.; Conte, P.; Guanieri, V.; Ficarra, G.; Mathieu, M.C.; Delaloge, S.; Curigliano, G.; et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: A retrospective multicenter study. Ann. Oncol. 2014, 25, 611–618; Erratum in Ann. Oncol. 2015, 26, 1518. [CrossRef] [PubMed]
3. Ibrahim, E.M.; Al-Heidei, M.E.; Al-Mansour, M.M.; Kazkaz, G.A. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: A meta-analysis. Breast Cancer Res. Treat. 2014, 148, 467–476. [CrossRef] [PubMed]
4. Loi, S.; Michiels, S.; Salgado, R.; Sirtaine, N.; Jose, V.; Fumagalli, D.; Kellokumpu-Lehtinen, P.L.; Bono, P.; Kataja, V.; Desmedt, C.; 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, 1544–1550. [CrossRef] [PubMed]
5. Narang, P.; Chen, M.; Sharma, A.A.; Anderson, K.S.; Wilson, M.A. The neoepitope landscape of breast cancer: Implications for immunotherapy. BMC Cancer 2019, 19, 200. [CrossRef]
6. Kim, Y.A.; Lee, H.J.; Heo, S.H.; Park, H.S.; Park, S.Y.; Bang, W.; Song, I.H.; Park, I.A.; Gong, G. Mxa expression is associated with tumor-infiltrating lymphocytes and is a prognostic factor in triple-negative breast cancer. Breast Cancer Res. Treat. 2016, 156, 597–606. [CrossRef]
7. Li, M.; Xu, J.; Jiang, C.; Zhang, J.; Sun, T. Predictive and prognostic role of peripheral blood t-cell subsets in triple-negative breast cancer. Front. Oncol. 2022, 12, 842705. [CrossRef]
8. Lundberg, A.; Li, B.; Li, R. B cell-related gene signature and cancer immunotherapy response. *Br. J. Cancer* **2022**, *126*, 899–906. [CrossRef]

9. Stovgaard, E.S.; Nielsen, D.; Hogdall, E.; Balslev, E. Triple negative breast cancer-prognostic role of immune-related factors: A systematic review. *Acta Oncol.* **2018**, *57*, 74–82. [CrossRef]

10. Yu, S.; Hu, C.; Liu, L.; Cai, L.; Du, X.; Yu, Q.; Lin, F.; Zhao, J.; Zhao, Y.; Zhang, C.; et al. Comprehensive analysis and establishment of a prediction model of alternative splicing events reveal the prognostic predictor and immune microenvironment signatures in triple negative breast cancer. *J. Transl. Med.* **2020**, *18*, 286. [CrossRef]

11. Zhang, Y.; Tian, J.; Qu, C.; Tang, Z.; Wang, Y.; Li, K.; Yang, Y.; Liu, S. Prognostic value of programmed cell death ligand-1 expression in breast cancer: A meta-analysis. *Medicine 2020*, *99*, e23359. [CrossRef] [PubMed]

12. Zhou, J.; Wang, X.H.; Zhao, Y.X.; Chen, C.; Xu, X.Y.; Sun, Q.; Wu, H.Y.; Chen, M.; Sang, J.F.; Su, L.; et al. Cancer-associated fibroblasts correlate with tumor-associated macrophages infiltration and lymphatic metastasis in triple negative breast cancer patients. *J. Cancer* **2018**, *9*, 4635–4641. [CrossRef] [PubMed]

13. Klintrup, K.; Makinen, J.M.; Kauppila, S.; Vare, P.O.; Melkko, J.; Tuominen, H.; Tuppurainen, K.; Makela, J.; Karttunen, T.J.; Makinen, M.J. Inflammation and hormone profile in colorectal cancer patients. *Eur. J. Cancer 2005*, *41*, 2645–2654. [CrossRef] [PubMed]

14. Ditsch, N.; Denkert, C.; Demaria, S.; Sirtaine, N.; Klauschen, F.; Pruneri, G.; Wiensert, S.; van den Eynden, G.; Baehner, F.L.; Penault-Llorca, F.; et al. The evaluation of tumor-infiltrating lymphocytes (tis) in breast cancer: Recommendations by an international tis working group 2014. *Ann. Oncol.* **2015**, *26*, 259–271. [CrossRef]

15. Khalique, S.; Nash, S.; Mansfield, D.; Wampfler, J.; Attygale, A.; Vroobel, K.; Kemp, H.; Buus, R.; Cottom, H.; Roxanis, I.; et al. Quantitative assessment and prognostic associations of the immune landscape in ovarian clear cell carcinoma. *Cancers 2021*, *13*, 3854. [CrossRef]

16. Leong, P.P.; Mohammad, R.; Ibrahim, N.; Ithnin, H.; Abdullah, M.; Davis, W.C.; Seow, H.F. Phenotyping of lymphocytes expressing regulatory and effector markers in infiltrating ductal carcinoma of the breast. *Immunol. Lett. 2006*, *102*, 229–236. [CrossRef]

17. Droesch, R.; Zlobec, I.; Kilic, E.; Guth, U.; Heberer, M.; Spagnoli, G.; Oertli, D.; Tapia, C. Differential pattern and prognostic significance of cd4+, fopx3+ and il-17+ tumor infiltrating lymphocytes in ductal and lobular breast cancers. *BMC Cancer 2012*, *12*, 134. [CrossRef]

18. Chan, M.S.; Wang, L.; Felizola, S.J.; Ueno, T.; Toi, M.; Loo, W.; Chow, L.W.; Suzuki, T.; Sasano, H. Changes of tumor infiltrating lymphocyte subtypes before and after neoadjuvant endocrine therapy in estrogen receptor-positive breast cancer patients—An immunohistochemical study of cd8+ and fopx3+ using double immunostaining with correlation to the pathobiological response of the patients. *Int. J. Biol. Markers 2012*, *27*, e295–e304. [CrossRef]

19. Boissiere-Michot, F.; Chabab, G.; Mollevi, C.; Guiu, S.; Lopez-Crapez, E.; Lopez-Crapez, E.; Ramos, J.; Bonnefoy, N.; Lafont, V.; Journet, P.J.; Makinen, M.J. Inflammation and hormone profile in colorectal cancer patients. *Eur. J. Cancer 2005*, *41*, 2645–2654. [CrossRef] [PubMed]

20. Leong, P.P.; Mohammad, R.; Ibrahim, N.; Ithnin, H.; Abdullah, M.; Davis, W.C.; Seow, H.F. Phenotyping of lymphocytes expressing regulatory and effector markers in infiltrating ductal carcinoma of the breast. *Immunol. Lett. 2006*, *102*, 229–236. [CrossRef]

21. Ditsch, N.; Mayr, D.; Lenhard, M.; Strauss, C.; Vodermaier, A.; Gallwas, J.; Stoeckl, D.; Graeser, M.; Weissbencher, T.; Friese, K.; et al. Correlation of thyroid hormone, retinoid x, peroxisome proliferator-activated, vitamin d and oestrogen/progesterone receptors in breast carcinoma. *Oncol. Lett. 2012*, *4*, 665–671. [CrossRef] [PubMed]

22. Dominguez, M.; Alvarez, S.; de Lera, A.R. Natural and structure-based rrx ligand scaffolds and their functions. *Curr. Top. Med. Chem. 2017*, *17*, 631–641. [CrossRef] [PubMed]

23. Dunlop, T.W.; Vaisanen, S.; Frank, C.; Molnar, F.; Sinkkonen, L.; Carlberg, C. The human peroxisome proliferator-activated receptor delta gene is a primary target of 1alpha,25-dihydroxyvitamin d3 and its nuclear receptor. *Reprod. Biol. Endocrinol.* **2005**, *3*, 898–915. [CrossRef] [PubMed]

24. Heublein, S.; Mayr, D.; Meindl, A.; Kircher, A.; Jeschke, U.; Ditsch, N. Vitamin d receptor, retinoid x receptor and peroxisome proliferator-activated receptor gamma expression in breast cancer: An immunohistochemical study. *Vivo 2012*, *26*, 87–92.
32. Heublein, S.; Mayr, D.; Meindl, A.; Angele, M.; Gallwas, J.; Jeschke, U.; Ditsch, N. Thyroid hormone receptors predict prognosis in brca1 associated breast cancer in opposing ways. *Plos ONE* 2015, 10, e0127072. [CrossRef] [PubMed]

33. Jeschke, U.; Zhang, X.; Kuhn, C.; Jalaguier, S.; Colinge, J.; Pfender, K.; Mayr, D.; Ditsch, N.; Harbeck, N.; Mahner, S.; et al. The prognostic impact of the aryl hydrocarbon receptor (ahr) in primary breast cancer depends on the lymph node status. *Int. J. Mol. Sci.* 2019, 20, 1016. [CrossRef]

34. Zahi Zehni, A.; Jacob, S.N.; Mummm, J.N.; Heidegger, H.H.; Ditsch, N.; Mahner, S.; Jeschke, U.; Vilsmaier, T. Hormone receptor expression in multicentric/multifocal versus unifocal breast cancer: Especially the vdr determines the outcome related to focality. *Int. J. Mol. Sci* 2019, 20, 5740. [CrossRef]

35. Shao, W.; Kuhn, C.; Jalaguier, S.; Kailuwait, M.; Wolf, V.; Harbeck, N.; Mahner, S.; Jeschke, U.; Cavailles, V.; et al. Cytoplasmic ppargamma is a marker of poor prognosis in patients with cox-1 negative primary breast cancers. *J. Transl. Med.* 2020, 18, 94. [CrossRef]

36. Shao, W.; Kuhn, C.; Mayr, D.; Ditsch, N.; Kailuwait, M.; Wolf, V.; Harbeck, N.; Mahner, S.; Jeschke, U.; Cavailles, V.; et al. Cytoplasmic and nuclear forms of thyroid hormone receptor beta1 are inversely associated with survival in primary breast cancer. *Int. J. Mol. Sci* 2020, 21, 330. [CrossRef]

37. Zehni, A.Z.; Batz, F.; Vattai, A.; Kaltofen, T.; Schrader, S.; Jacob, S.N.; Mummm, J.N.; Heidegger, H.H.; Ditsch, N.; Mahner, S.; et al. The prognostic impact of retinoid x receptor and thyroid hormone receptor alpha in unifocal vs. Multifocal/multicentric breast cancer. *Int. J. Mol. Sci* 2021, 22, 957. [CrossRef]

38. Shao, W.; Kuhn, C.; Mayr, D.; Ditsch, N.; Kailuwait, M.; Wolf, V.; Harbeck, N.; Mahner, S.; Jeschke, U.; Cavailles, V.; et al. Cytoplasmic lxr expression is an independent marker of poor prognosis for patients with early stage primary breast cancer. *J. Cancer Res. Clin. Oncol.* 2021, 147, 2535–2544. [CrossRef]

39. Shao, W.; Kopke, M.B.; Vilsmaier, T.; Zehni, A.Z.; Kessler, M.; Sixou, S.; Schneider, M.; Ditsch, N.; Cavailles, V.; Jeschke, U. Cytoplasmic colocalization of rxralpha and ppargamma as an independent negative prognosticator for breast cancer patients. *Cells* 2022, 11, 1244. [CrossRef]

40. Zahi Zehni, A.; Batz, F.; Cavailles, V.; Sixou, S.; Kaltofen, T.; Keckstein, S.; Heidegger, H.H.; Ditsch, N.; Mahner, S.; Jeschke, U.; et al. Cytoplasmic localization of rxralpha determines outcome in breast cancer. *Cancers* 2021, 13, 3756. [CrossRef]

41. Elston, E.W.; Ellis, I.O. Method for grading breast cancer. *J. Clin. Pathol.* 1993, 46, 189–190. [CrossRef] [PubMed]

42. Ditsch, N.; Heublein, S.; Jeschke, U.; Sattler, C.; Kuhn, C.; Hester, A.; Czogalla, B.; Trillsch, F.; Mahner, S.; Engel, J.; et al. Cytoplasmic versus nuclear thr alpha expression determines survival of ovarian cancer patients. *J. Cancer Res. Clin. Oncol.* 2020, 146, 1923–1932. [CrossRef]

43. Von Kneathen, A.; Tzielpy, N.; Jennewein, C.; Brüne, B. Casein-kinase-ii-dependent phosphorylation of ppargamma provokes crm1-mediated shuttling of ppargamma from the nucleus to the cytosol. *J. Cell Sci.* 2010, 123, 192–201. [CrossRef] [PubMed]

44. Lee, H.J.; Lee, J.I.; Song, I.H.; Park, I.A.; Kang, J.; Yu, J.H.; Ahn, J.H.; Gong, G. Prognostic and predictive value of nanostring-based immune-related gene signatures in a neoadjuvant setting of triple-negative breast cancer: Relationship to tumor-infiltrating lymphocytes. *Breast Cancer Res. Treat.* 2015, 151, 619–627. [CrossRef]

45. Wang, K.; Shen, T.; Siegal, G.P.; Wei, S. The cd4/cd8 ratio of tumor-infiltrating lymphocytes at the tumor-host interface has prognostic value in triple-negative breast cancer. *Hum. Pathol.* 2017, 69, 110–117. [CrossRef]

46. Gao, G.; Wang, Z.; Qu, X.; Zhang, Z. Prognostic value of tumor-infiltrating lymphocytes in patients with triple-negative breast cancer: A systematic review and meta-analysis. *BMC Cancer* 2020, 20, 179. [CrossRef]

47. Kuroda, H.; Jamiyan, T.; Yamaguchi, R.; Kakumoto, A.; Abe, A.; Harada, O.; Enkhbat, B.; Masunaga, A. Prognostic value of tumor-infiltrating lymocytes and plasma cells in triple-negative breast cancer. *Breast Cancer* 2021, 28, 904–914. [CrossRef]

48. De Jong, V.M.T.; Wang, Y.; Hoeve, N.D.T.; Opdam, M.; Stathonikos, N.; Jozwiak, K.; Hauptmann, M.; Cornelissen, S.; Vreuls, W.; Rosenberg, E.H.; et al. Prognostic value of stromal tumor-infiltrating lymphocytes in young, node-negative, triple-negative breast cancer patients who did not receive (neo)adjuvant systemic therapy. *J. Clin. Oncol.* 2020, 42, 2361–2374. [CrossRef]

49. Mohammed, A.A.; Elsayed, F.M.; Algazar, M.; Rashed, H.E. Predictive and prognostic value of tumor- infiltrating lymphocytes for pathological response to neoadjuvant chemotherapy in triple negative breast cancer. *Gulf J. Oncol.* 2022, 1, 53–60.

50. Desmedt, C.; Salgado, R.; Fornili, M.; Pruneri, G.; van den Eynden, G.; Zoppoli, G.; Rothe, F.; Buissere, L.; Garaud, S.; Willard-Gallo, K.; et al. Immune infiltration in invasive lobular breast cancer. *J. Natl. Cancer Inst.* 2018, 110, 768–776. [CrossRef] [PubMed]

51. Savas, P.; Sędziwia, R.; Denkert, C.; Sotiriou, C.; Darcy, P.K.; Smyth, M.J.; Loi, S. Clinical relevance of host immunity in breast cancer: From tils to the clinic. *Nat. Rev. Clin. Oncol.* 2016, 13, 228–241. [CrossRef]

52. Pruneri, G.; Vingiani, A.; Denkert, C. Tumor infiltrating lymphocytes in early breast cancer. *Breast* 2018, 37, 207–214. [CrossRef]

53. Honda, C.; Kurozumi, S.; Katayama, A.; Hanna-Khalil, B.; Masuda, K.; Nakazawa, Y.; Ogino, M.; Obayashi, S.; Yamaji, R.; Makiguchi, T.; et al. Prognostic value of tumor-infiltrating lymphocytes in estrogen receptor-positive and human epidermal growth factor receptor 2-negative breast cancer. *Mol. Clin. Oncol.* 2021, 15, 252. [CrossRef]

54. Criscitiello, C.; Vingiani, A.; Maisonneuve, P.; Viale, G.; Viale, G.; Curigliano, G. Tumor-infiltrating lymphocytes (tils) in er+/her2-breast cancer. *Breast Cancer Res. Treat.* 2020, 183, 347–354. [CrossRef]

55. He, J.; Fu, F.; Wang, W.; Xi, G.; Guo, W.; Zheng, L.; Ren, W.; Qiu, L.; Huang, X.; Wang, C.; et al. Prognostic value of tumour-infiltrating lymphocytes based on the evaluation of frequency in patients with oestrogen receptor-positive breast cancer. *Eur. J. Cancer* 2021, 154, 217–226. [CrossRef] [PubMed]
56. Scholz, C.; Toth, B.; Barthell, E.; Mylonas, I.; Weissenbacher, T.; Friese, K.; Jeschke, U. Immunohistochemical expression of glycodelin in breast cancer correlates with estrogen-receptor alpha and progesterone-receptor a positivity. *Histol. Histopathol.* 2009, 24, 467–471. [CrossRef]

57. Thorpe, S.M.; Rose, C.; Rasmussen, B.B.; King, W.J.; DeSombre, E.R.; Blough, R.M.; Mouridsen, H.T.; Rossing, N.; Andersen, K.W. Steroid hormone receptors as prognostic indicators in primary breast cancer. *Breast Cancer Res. Treat.* 1986, 7, S91–S97.

58. Thorpe, S.M.; Rose, C.; Rasmussen, B.B.; Mouridsen, H.T.; Bauer, T.; Keiding, N. Prognostic value of steroid hormone receptors: Multivariate analysis of systemically untreated patients with node negative primary breast cancer. *Cancer Res.* 1987, 47, 6126–6133.

59. Loi, S.; Dushyanthen, S.; Beavis, P.A.; Salgado, R.; Denkert, C.; Savas, P.; Combs, S.; Rimm, D.L.; Gillman, J.M.; Estrada, M.V.; et al. Ras/mapk activation is associated with reduced tumor-infiltrating lymphocytes in triple-negative breast cancer. Therapeutic cooperation between mek and pd-1/pd-1 immune checkpoint inhibitors. *Clin. Cancer Res.* 2016, 22, 1499–1509. [CrossRef] [PubMed]

60. Patra, S.; Elahi, N.; Armarer, A.; Arunachalam, S.; Omala, J.; Hamid, I.; Ashton, A.W.; Joyce, D.; Jiao, X.; Pestell, R.G. Mechanisms governing metabolic heterogeneity in breast cancer and other tumors. *Front. Oncol.* 2021, 11, 700629. [CrossRef] [PubMed]

61. Hernandez-Quiles, M.; Broekema, M.F.; Kalkhoven, E. Ppargamma in metabolism, immunity, and cancer: Unified and diverse mechanisms of action. *Front. Endocrinol. (Lausanne)* 2021, 12, 624112. [CrossRef]

62. Chekaoui, A.; Ertl, H.C.J. Pparalpha agonist fenofibrate enhances cancer vaccine efficacy. *Cancer Res.* 2021, 81, 4431–4440. [CrossRef] [PubMed]

63. Hichami, A.; Yessoufou, A.; Ghiringhelli, F.; Salvadori, F.; Moutairou, K.; Zwetyenga, N.; Khan, N.A. Peroxisome proliferator-activated receptor alpha deficiency impairs regulatory t cell functions: Possible application in the inhibition of melanoma tumor growth in mice. *Biochimie* 2016, 131, 131. [CrossRef] [PubMed]

64. Zhang, Y.; Kurupati, R.; Liu, L.; Zhou, X.Y.; Zhang, G.; Huda, I.; Filisio, F.; Giles-Davis, W.; Xu, X.; Karakousis, G.C.; et al. Enhancing cd8(+) t cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. *Cancer Cell* 2017, 32, 377–391.e9. [CrossRef] [PubMed]