Towards predictive biomarkers for immunotherapy response in breast cancer patients

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Practice points

- Although response rates to anti-PD(L)1 are moderate in breast cancer, durable responses are seen.
- No accurate biomarkers for the prediction of response to immunotherapy in breast cancer are available yet.
- Response rates to anti-PD(L)1 appear higher in triple-negative breast cancer (TNBC) and PDL1-positive breast cancer.
- Early data show that TNBC patients with a tumor harboring relatively high levels of tumor infiltrating lymphocytes have a better outcome after anti-PD(L)1.
- As seen in melanoma patients, TNBC patients with high serum lactate dehydrogenase levels are less likely to respond to anti-PD(L)1 monotherapy.
- Exploiting research on immune biomarkers in other cancer types can guide the analyses of samples of breast cancer patients treated with anti-PD(L)1 in order to find predictive biomarkers.
- It needs to be determined whether potential biomarkers predicting response to anti-PD(L)1 have solely predictive value or capture information on prognosis as well.

Immunotherapy using anti-PD(L)1 has revolutionized treatment for various tumor types. Early data have shown durable responses in a small subgroup of breast cancer patients. So far, the response rates appear higher for breast tumors that are triple negative, PDL1-positive and/or harbor high levels of immune cells. Both comprehensive analyses of the breast tumor microenvironment and exploiting research on biomarkers in other cancer types, such as melanoma and lung cancer, may contribute to the discovery of accurate biomarkers to select breast cancer patients for immunotherapy. Here we summarize key features of the breast tumor microenvironment as well as putative predictive biomarkers established in other tumor types. Insights from both fields can guide future studies to enable personalized breast cancer immunotherapy.

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Immunotherapy has revolutionized cancer treatment for various tumor types. The major breakthroughs are seen with antagonistic monoclonal antibodies directed against CTLA-4, PD-1 and PD-L1. Objective response rates (ORRs) with anti-PD(L)1 treatment range from 40% in stage IV melanoma patients [1] to 19–23% in stage IV non-small-cell lung cancer (NSCLC) [2–4] and 25% in renal cell carcinoma (RCC) patients [5]. Importantly, durable responses are seen in patients responding to therapy. Recently, impressive progression-free survival (PFS) benefits of anti-PDL1 were seen in stage III NSCLC patients after chemoradiotherapy as compared with placebo [6] and longer recurrence-free survival with adjuvant anti-PD1 in stage III/IV melanoma as compared with anti-CTLA-4 [7].

Historically, breast cancer was long considered a low-immunogenic malignancy. However, accumulating data regarding the value of breast tumor-associated immune cells and early data on efficacy of immune checkpoint inhibition such as anti-PD(L)1 in metastatic breast cancer have led to reconsideration of this viewpoint. Anti-PD(L)1 is currently under investigation in breast cancer patients and promising response rates have been presented. In order to treat the right patients with the right immunomodulatory therapy, biomarkers are needed to predict

Figure 1. Opportunities for development of biomarkers to predict immunotherapy response in breast cancer.

Currently, ongoing analyses of samples of breast cancer patients treated with anti-PD(L)1 have shown that ER-status, TIL, CD8 and LDH could be potential biomarkers (middle panel). New biomarker analyses can be guided by mechanism based preclinical or translational research (left panel) or from clinical trials with anti-PD(L)1 in other cancers, such as melanoma, NSCLC or bladder cancer (right panel). Information on these markers and references can be found in the text.

[†]US FDA approved biomarkers (PD-L1: ≥50% expression on tumor cells in NSCLC).

BC: Breast cancer; DC: Dendritic cell; ER: Estrogen receptor; ESR: Erythrocyte sedimentation rate; LDH: Lactate dehydrogenase; MDSC: Myeloid-derived suppressor cell; MMR: Mismatch repair; NLR: Neutrophil-to-lymphocyte ratio; NSCLC: Non-small-cell lung cancer; TAM: Tumor-associated macrophage; TCR: T-cell receptor; T_{eff}: Effector T-cell; TIL: Tumor infiltrating lymphocyte; TME: Tumor microenvironment; T_{regs}: Regulatory T-cell.

response to immunotherapy. For breast cancer, no predictive biomarkers for immunotherapy are available yet. In-depth analyses of the breast cancer tumor microenvironment (TME) and the systemic immune response of breast cancer patients may help the identification of possible biomarkers. Furthermore, putative biomarkers found in other malignancies can guide the discovery of predictive biomarkers in breast cancer. In this review, we will describe the current status of biomarker research based on the small clinical trials evaluating anti-PD(L)1 in metastatic breast cancer presented so far. In addition, we summarize previous preclinical and translational research on the anti-breast cancer immune response that might be crucial for response to anti-PD(L)1. Lastly, we will discuss putative predictive biomarkers established in other tumor types that might be important for the selection of breast cancer patients for immunotherapy. Figure 1 summarizes the opportunities for the development of biomarkers to predict immunotherapy response in breast cancer.

First results of trials evaluating anti-PD(L)1 in breast cancer

In 170 patients with triple-negative breast cancer (TNBC), pretreated with chemotherapy for metastatic disease and not preselected based on PD-L1 expression (KEYNOTE-086A), an ORR of 5% was found upon treatment

with anti-PD1 (pembrolizumab) [8]. In another cohort (KEYNOTE-012) of 27 patients with heavily pretreated PD-L1-positive metastatic TNBC (mTNBC), the ORR was 19% [9]. In the KEYNOTE-86B cohort of 52 patients treated with anti-PD1 as first line of therapy for metastatic disease and with PD-L1-positive tumors, the ORR was 23% [10]. In another Phase I study with 112 patients with mTNBC, treated with anti-PDL1 (atezolizumab), an ORR of 10% was seen [11]. In conclusion, in mTNBC response rates presented so far are around 5–23% and seem to be associated with PD-L1 expression and extend of previous chemotherapies.

On a smaller scale, anti-PD(L)1 has been evaluated in metastatic ER-positive disease. Dirix *et al.* found an ORR of 3% in 72 patients after treatment with anti-PDL1 (avelumab) [12]. The KEYNOTE-028 study with 25 ER-positive metastatic breast cancer patients, all with PD-L1-positive tumors, revealed an ORR of 12% [13]. Currently, many trials are ongoing in metastatic breast cancer evaluating the role of anti-PD(L)1 in combination with chemotherapy. Anti-PDL1 plus nab-paclitaxel resulted in an ORR of 42% in 24 mTNBC patients [14]. The combination of eribulin and anti-PD1 in 39 pretreated mTNBC patients induced responses in 33% of the patients [15]. It still remains unclear whether this potential synergy between anti-PD(L)1 and chemotherapy is simply related to the direct effect on tumor growth or to proposed immunomodulatory aspects of chemotherapy [16]. Preliminary data presented at ESMO 2017 of the Phase II TONIC trial (NCT02499367) showed an ORR of 24% in 50 mTNBC patients after 2 weeks of pretreatment with either low-dose chemotherapy (cisplatin, doxorubicin and cyclophosphamide), radiotherapy or no pretreatment, followed by anti-PD1 (nivolumab) [17]. In-depth analyses of tumor biopsies are needed to unravel the potential immunomodulatory effect of chemotherapy/radiation on the breast TME.

Several neoadjuvant immunotherapy trials are ongoing in early breast cancer patients. In the TNBC cohort of the neoadjuvant adaptive I-SPY trial, Nanda *et al.* found three-times higher estimated complete response rates when combining anti-PD1 with paclitaxel, compared with paclitaxel monotherapy; 60 versus 20%. Furthermore, the estimated complete response rate was significantly increased in ER-positive disease with the addition of anti-PD1 (34 vs 13%) [18]. Schmid *et al.* described an ORR of 100% in ten early TNBC patients treated with carboplatin/nab-paclitaxel plus anti-PD1, followed by doxorubicin/cyclophosphamide (AC) and anti-PD1 [19]. Lastly, preliminary data in a third trial showed a pathological complete response rate of 71% in seven early TNBC patients treated with anti-PDL1 (durvalumab) plus nab-paclitaxel and AC [20].

Potential biomarkers predicting outcome of anti-PD(L)1 in breast cancer

As described above, so far, a limited number of patients were enrolled in clinical trials evaluating anti-PD(L)1 in breast cancer. Moreover, these trials are not designed for the discovery and validation of biomarkers. In two studies PD-L1-positive TNBC had higher ORR as compared with patients with low expression of PD-L1 [11,12]. Furthermore, patients with at least 10% stromal tumor infiltrating lymphocytes (sTIL) or 1.35% CD8-positive cells (median used as cut-off) tended to have higher response rates [11]. Recently, Loi *et al.* confirmed the predictive value of sTIL in the KEYNOTE-086 cohorts, in which significantly higher levels of sTIL were seen in mTNBC patients responding to anti-PD1. This effect was even more pronounced in the KEYNOTE-86B cohort of PDL1-positive tumors that were not pretreated for metastatic disease. Of note, PD-L1 expression itself was not correlated with outcome after anti-PD1 [21].

Only a few studies have reported on potential biomarkers using peripheral blood. In the KEYNOTE-012 study, all five patients with a serum lactate dehydrogenase (LDH) level of above 800 U/l progressed within 8 weeks after study entry [9]. Also in the KEYNOTE-086 cohorts, LDH was associated with worse outcome after anti-PD1 treatment [21]. So far, no data are available on the predictive capacity of genetic characteristics in breast cancer, such as mutational load and gene expression. However, a slightly higher neoantigen load was seen in responders to combination therapy (anti-PDL1 plus anti-CTLA-4) in a small cohort of mTNBC and ER-positive patients [22].

Breast tumor microenvironment

Besides future unbiased analyses of side studies from the trials summarized above, insights gained in the last decades from preclinical as well as translational breast cancer research may be helpful on our way to personalized breast cancer immunotherapy. Here we discuss the most important components of the breast TME that could inform future analyses of samples of patients treated with anti-PD(L)1.

Tumor intrinsic features

Genomic instability is a hallmark of cancer and an important determinant of the immunogenicity of cancer cells. Accumulation of tumor-specific nonsynonymous mutations, called mutational load, can lead to the formation

of peptides that are entirely absent from the normal human genome, so-called neoantigens. This can increase the 'foreignness' of the tumor and can thereby evoke an immune response [23]. In breast cancer, HER2-enriched tumors and TNBC exhibit a significantly higher mutation rate than ER-positive disease and are therefore more likely to harbor more neoantigens [24,25]. Copy number variants are caused by stable chromosomal rearrangements and occur early in breast cancer [26]. BRCA1/2-deficient breast cancers have impaired DNA double-strand break repair, which causes profuse copy number variant formation [27,28]. *BRCA1*-mutated cancers are associated with more lymphocytic infiltration as compared with a sporadic breast cancer control group [29]. Recently, Nolan *et al.* confirmed that TNBC tumors in the presence of a germline *BRCA1* mutation have more sTIL and higher PD-L1 expression, as compared with wild-type tumors [30]. In addition, in DNA damage response-deficient tumors, more infiltration of CD4⁺ and CD8⁺ T cells was seen [31].

Recently, it has been shown that response rates to anti-PD1 are strikingly high in mismatch repair deficient (MMRd) tumors independent of the origin of the cancer cell [32]. Microsatellite instability, caused by MMRd, results in rapid accumulation of somatic mutations. MMRd is rare in breast cancer, but is present in about 1–2% of cases [33]. Profound response to anti-PD1 was seen in a breast cancer patient with a MMRd tumor [34]. Importantly, microsatellite instability might be associated with lower survival rates as seen in a small cohort of breast cancer patients [35].

Tumor infiltrating lymphocytes

Considerable heterogeneity exists between mammary tumors with respect to tumor infiltrating lymphocyte (TIL) density and immune cell composition [36]. Higher lymphocyte infiltration is correlated with ER negativity and better prognosis in general [37,38]. Moreover, high TIL levels have been associated with improved responses to neoadjuvant chemotherapy [39]. A distinction can be made between intratumoral and stromal lymphocytes. Stromal lymphocytes are considered a more reliable biomarker than intratumoral lymphocytes, probably due to the poor reproducibility of intratumoral TIL [40]. The lymphocyte population in breast cancers is largely comprised of CD8⁺ cytotoxic T cells and CD4⁺ T-helper cells, followed by regulatory T cells and different subpopulations of B cells [41,42]. High CD8⁺ T-cell infiltration is a positive prognostic factor generally associated with TNBC and HER2-enriched lesions [43]. Different subpopulations of CD4⁺ T cells are present in the breast cancer TME, which include T-helper (Th) subsets, regulatory T cells (T_{regs}), $\gamma\delta$ T cells and follicular T cells [44,45]. The presence of Th17 cells in breast cancer was associated with good prognosis, although functionally both pro- and antitumor functions have been reported [46]. T_{regs} are CD4⁺ CD25⁺ FOXP3⁺ T cells with a crucial role in protecting tissues from damage due to inflammation and preventing autoimmunity [47]. The presence of T_{regs} is negatively correlated with disease outcome in breast cancer [48]. A small subset of T cells is represented by the $\gamma\delta$ T cells, which have an alternative T-cell receptor (TCR) that is able to recognize antigens in MHC-unrestricted fashion [49]. Like T_{regs}, these $\gamma\delta$ T cells exhibit a regulatory function and were correlated to advanced stages and worse survival in breast cancer [50,51]. Conversely, follicular T cells have an antitumor role since they were associated with better prognosis in HER2-positive breast cancer [45]. Tumor-reactive B cells can produce antibodies that recognize neoantigen epitopes, which potentiates tumor elimination by T cells and innate immune cells [52]. Tumor-evoked regulatory B cells (B_{regs}) were shown to secrete immunosuppressive cytokines, which inhibited proliferation of CD4⁺ T cells. In addition, PD-L1 expression on B cells was shown to inhibit antitumor T-cell responses [53].

Tumor infiltrating myeloid cells

Tumor-associated macrophages (TAMs) are known to facilitate metastasis [54,55]. In breast cancer, TAMs have been associated with ER negativity, higher histological grade and poor prognosis [56]. Neutrophils reduce T-cell proliferation and therefore suppress effector function. Alongside $\gamma\delta$ T cells, neutrophils have a role in promoting breast cancer metastasis [57]. In a mechanistic mouse study, it was shown that inflamed mammary tumors secrete chemokine IL-1 β , which attracts $\gamma\delta$ T cells and induces them to produce anti-inflammatory cytokine IL-17 and G-CSF. Neutrophils are recruited by these factors and secrete inducible NOS, which in turn suppresses antitumor CD8⁺ T-cell effector function and therefore facilitates metastasis [57]. Lastly, myeloid-derived suppressive cells (MDSCs) are immature myeloid cells that exhibit immunosuppressive capacity. MDSCs inhibit effector functions of T cells, natural killer (NK) cells and dendritic cells (DCs), while stimulating protumor functions of T_{regs}, TAMs and Th2 cells through various mechanisms. Infiltrating MDSCs have been associated with unfavorable outcome in breast cancer [58]. Of note, new insights concerning tumor infiltrating myeloid cells have also generated confusion due to, for example, the lack of consensus on phenotypic markers to detect polarized macrophages or MDSCs [59].

Other features of immunosuppression in breast cancer

A balance between stimulatory and inhibitory signals regulates T-cell activity. One of the most important immune checkpoints in peripheral immunological tolerance is governed by PD-1. This co-inhibitory immune receptor represses the effector function of cytotoxic T cells to prevent autoimmunity [60]. PD1 expression is induced on T cells upon TCR activation [61,62]. Besides activated T cells, also activated B cells, NK cells and several other immune lineages were shown to express PD1 [63]. PD-L1, also known as B7-H1, is broadly expressed among different populations of immune cells and certain tissues, whereas PD-L2 is mainly limited to DCs. Cytokine IFN- γ , secreted by activated T cells, is shown to induce expression of both PD-L1 and PD-L2 [64,65]. Remarkably, in breast cancer PD-L1 expression was mainly observed on immune cells as opposed to tumor cells in other cancers [66].

IFN- γ secreted in the TME induces expression of IDO [67]. IDO was linked to high TIL density in breast cancer [68]. Conversely, in a breast cancer murine model, high IDO expression was reported to have an immunosuppressive function on T cells, and might interfere with immunotherapy efficacy [69]. IDO inhibition is currently being evaluated in Phase I clinical trials in combination with immune checkpoint blockade [70]. Lastly, CD73 is upregulated by T_{regs} as another immune escape mechanism [71]. As a result of enzymatic activity of CD73, the A2a receptor (A2aR) on T cells is activated, which in turn enhances PD1 expression [72,73]. CD73 expression has been associated with poor outcome in TNBC [74]. Both CD73 and A2aR antagonist therapies synergize with PD1 blockade in mouse models [73,75].

Systemic immune response in breast cancer

In contrast to chemotherapy or targeted therapy, immunotherapy is not directed against the cancer cells but can modulate the patient's immune system. Emerging data in the field of clinical melanoma research show that circulating immunosuppressive cells or factors are associated with poor outcome after immunotherapy. Here we summarize the current knowledge on features of this systemic immune response in breast cancer.

Circulating immune cells

It was shown that breast cancer patients have decreased peripheral lymphocyte levels, but differences were only statistically significant for CD8⁺ and CD4⁺ T cells [76]. Compared with healthy controls, higher peripheral blood T_{reg} counts were found in breast cancer patients [77,78]. Elevated neutrophil-to-lymphocyte ratio as a measure for systemic inflammation has recently been associated with lower survival rates [79]. Peripheral blood DC counts, myeloid-derived DCs in particular, were shown to be consistently reduced in breast cancer patients [80]. In advanced breast cancer patients, DC count was dramatically lower as compared with early breast cancer, where more immature DCs were observed [80,81]. Finally, PD-L1 mRNA expression in immune cells detected in peripheral blood might be associated with disease progression [82]. Interestingly, circulating metastatic breast cancer cells expressing PD-L1 were also detectable [83].

Secreted immunological mediators

In cancer patients, predominance of Th2-related cytokines was observed in peripheral blood, whereas Th1 cytokines were decreased [84]. Significantly reduced levels of IFN- γ (Th1 associated) were measured in breast cancer patients, suggesting that IFN- γ production by lymphocytes is partially or completely impaired [76]. Arginase serum levels were found to be elevated and increased during disease progression in breast cancer patients [85]. Treatment with VEGF antagonist bevacizumab inhibited the infiltration of suppressive immune cells (i.e., T_{regs}, TAMs and MDSCs) in a breast cancer xenograft model [86]. As discussed above, serum LDH levels are associated with outcome after anti-PD1 in TNBC, but LDH does also correlate with disease outcome in cancer. This suggests that LDH might not be a pure predictive factor for breast cancer immunotherapy response, but also captures prognostic information [87]. Acidification of the TME, as a result of conversion of pyruvate into lactate by LDH, has detrimental consequences for infiltrating T cells, such as impaired cytokine production and lytic activity [88].

Putative biomarkers for immunotherapy in other malignancies

Research in melanoma, bladder cancer and NSCLC has already given important insights into features of the TME that predict response to anti-PD(L)1. This information can be exploited with the aim to identify putative predictive biomarkers in breast cancer. According to the 'cancer immunogram' presented by Blank *et al.*, seven parameter classes can be used to describe the interaction between cancer and the immune system [89]. In this section, possible biomarkers found in other malignancies than breast cancer, will be described according to these classes.

Tumor foreignness

In patients with MMRd tumors treated with anti-PD1, high response rates are seen, independent of cancer type [32]. This led to the US FDA approval of MMRd as a biomarker for immunotherapy.

A relationship was shown between high nonsynonymous mutation burden and improved response to anti-PD1 in NSCLC patients [90]. The significance of mutational load as a predictive marker was also confirmed in bladder cancer [91]. Frameshift indel mutations, small insertions or deletions, were associated with better response to checkpoint inhibition in melanoma [92]. Furthermore, poor outcome after anti-CTLA-4 treatment was seen in patients with melanoma with tumor aneuploidy and higher burden of copy number loss [93,94]. Clonal neoantigens but not the subclonal mutations were predictive of response to anti-PD1 in NSCLC [95].

General immune status

Increases in peripheral blood lymphocyte count during treatment has been correlated to improved anti-CTLA-4 response, whereas a high neutrophil-to-lymphocyte ratio was associated with inferior outcome in anti-PD1-treated NSCLC patients [96,97]. Higher rates of MDSCs were associated with lower response rates in anti-CTLA-4 refractory melanoma patients treated with anti-PD1 [98] and lower overall survival (OS) in melanoma patients treated with anti-CTLA-4 [99]. In addition, it was seen that responders to anti-CTLA-4 had a higher increase of eosinophils after one infusion as compared with non-responders. Also, monocyte and neutrophil count at baseline were significantly lower in responders. A decrease in monocytic MDSCs, a reduction in PD-L1 expression on MDSCs seemed predictive of response to anti-CTLA-4 in the same cohort [100].

Immune cell infiltration

Increased TILs and CD8⁺ T cells in particular have been linked with therapeutic efficacy of PD(L)1 checkpoint blockade in different cancer types, especially in melanoma [101,102]. An increase in TIL after treatment and higher baseline levels of FOXP3 and IDO were associated with better clinical outcome in melanoma patients treated with anti-CTLA-4 [103]. Higher frequencies of FOXP3⁺ CD4⁺ T cells were also associated with better survival in melanoma patients treated with anti-CTLA-4 [104]. CTLA4 is known to be expressed on FOXP3⁺ T_{regs} and therefore might be important for anti-CTLA-4 efficacy [105]. FOXP3 in relation to response to anti-PD(L)1 has not been well described. In RCC patients treated with anti-PDL1 a subtle higher ratio of effector T cells to regulatory T cells was seen in responders, as measured by gene expression [106].

T-cell checkpoints

PD-L1 expression on tumors has been linked with better responses to immunotherapy across different cancers. However, PD-L1 expression does not appear to be a requisite for immunotherapeutic responses and PDL1-positive tumors are not always responsive [107,108]. Interestingly in some tumor types, such as bladder cancer, PD-L1 expression on immune cells appears to be more predictive of anti-PD(L)1 response than its expression on tumor cells [108,109], as might be the case for breast cancer.

Soluble inhibitors

Low baseline levels of the erythrocyte sedimentation rate were associated with better OS in melanoma patients treated with anti-CTLA-4 [110]. In a small cohort of 40 melanoma patients, high IL-6 levels were associated with worse outcome after anti-CTLA-4 treatment [96].

Inhibitory tumor mechanisms

LDH has been shown to inhibit function and survival of T cells and NK cells [111]. High levels of serum LDH are correlated with worse response to anti-CTLA-4 in melanoma patients [110]. Furthermore, higher response rates were seen in melanoma patients with normal LDH treated with anti-PD1 [112]. Whether LDH is mainly a prognostic factor or also predictive for immunotherapy response needs to be further established.

Immune infiltrate activation

T-effector- and IFN- γ -associated genes were associated with better OS in NSCLC patients treated with anti-PDL1 [113]. This was also shown in melanoma patients that responded to anti-CTLA-4 or anti-PD1, in which higher expression levels of genes were found related to CD8⁺ T cells, $\gamma\delta$ T and NK cells, and lower levels of genes associated with MDSCs and T_{regs} [114]. Higher IFN- γ associated gene expression levels were associated with better

response to anti-PDL1 in urothelial cell carcinoma and anti-CTLA-4 in melanoma [108,115]. Deep sequencing of the T-cell receptor in peripheral blood samples of melanoma patients showed a correlation between TCR repertoire and better OS after immune checkpoint blockade [116]. An increased clinical benefit to anti-CTLA-4 was observed in patients with higher baseline TCR diversity measured from peripheral blood of melanoma patients [117]. In addition, it was found that melanoma patients who responded to neoadjuvant anti-PD1 and anti-CTLA-4, had more newly detectable TCR clones after 6 weeks of treatment [118].

Gut microbiome

Accumulating evidence indicates that an overgrowth or absence of specific gut bacteria might predict response to immunotherapy. In mice with melanoma, *Bifidobacterium* was needed for anti-PDL1 response. After oral administration of this gut microbiome bacterium, tumors were reduced in size to the same extent as with anti-PDL1 alone [119]. In patients with metastatic melanoma treated with anti-PD1, response was associated with a 'favorable' gut microbiome. More diversity, high abundance of *Faecalibacterium* and low abundance of *Bacteroidales* was associated with response to anti-PD1 and longer PFS. Fecal microbiome transplant of germ-free mice with feces obtained from melanoma patients responding to anti-PD1, resulted in reduced tumor growth [120]. Another study showed that NSCLC and RCC patients treated with antibiotics during the course of anti-PD1 had shorter PFS and/or OS, as compared with patients not treated with antibiotics. This effect remained statistically significant in a multivariate analysis including other prognostic markers. An association between the abundance of *Akkermansia muciniphila* and a favorable clinical outcome was seen. Fecal microbiome transplant with *A. muciniphila* in sarcoma bearing mice restored response to anti-PD1 after treatment with antibiotics [121].

Future perspective

Knowledge on the breast TME, the systemic immune response in breast cancer patients and biomarkers found in other malignancies can guide the search for new biomarkers in breast cancer. In addition, understanding the mechanisms of response to immunotherapy in breast cancer will be crucial to increase benefit, including cure and quality of life of our breast cancer patients. To obtain this knowledge, comprehensive analysis of different types of data is essential. Therefore, large scale acquisition of cancer tissue, peripheral blood and other biospecimens is critical for the development of a robust classifier that can be used in the clinic to predict response and toxicity of immunotherapy in early and metastatic breast cancer patients.

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