Predictive biomarkers of response to immunotherapy in triple-negative breast cancer – state of the art and future perspectives

Prediktívne biomarkery v imunoterapii triple-negatívneho karcinómu prsníka – súčasné poznatky a perspektívy

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Summary

Background: Immunotherapy by using immune checkpoint inhibitors (ICIs) heralded a new era in the treatment of patients with advanced triple-negative breast cancer (TNBC). Nevertheless, in a substantial proportion of TNBC patients, the clinical outcomes of ICIs treatment remain unpredictable and proper biomarkers to identify tumors sensitive to immunotherapy are urgently needed. Currently, the most clinically relevant biomarkers used to predict efficacy of ICIs in patients with advanced TNBC remain the immunohistochemical analysis of programmed death-ligand 1 (PD-L1) expression, the assessment of tumor infiltrating lymphocytes (TILs) present in the tumor microenvironment (TME), and the evaluation of the tumor mutational burden (TMB). Emerging biomarkers related to activation of the transforming growth factor beta signaling pathway, the discoidin domain receptor 1, and thrombospondin-1 as well as other cellular and molecular factors present within TME, have the potential to be utilized as predictors of response to ICIs in the future. ***Purpose:*** In this review, we summarize the current knowledge of mechanisms regulating PD-L1 expression, of the predictive value of TILs as well as of associated cellular and molecular components present in the TME in TNBC. Furthermore, TMB and emerging biomarkers with potential value in predicting efficacy of ICIs are discussed, and new therapeutic strategies will be outlined.

Key words

triple-negative breast cancer – immunotherapy – programmed death-ligand 1 – tumor infiltrating lymphocytes – tumor mutational burden

Súhrn

Východiská: Imunoterapia s využitím inhibítorov imunitných kontrolných bodov (immune checkpoint inhibitors – ICIs) ohlásila novú éru v liečbe pokročilého triple-negatívneho karcinómu prsníka (TNBC). Avšak v značnej časti pacientov s TNBC je klinický dopad liečby ICIs nepredvídateľný a vhodné biomarkery identifikujúce nádory citlivé na imunoterapiu sú veľmi potrebné. V súčasnosti klinicky najviac relevantné prediktívne biomarkery účinnosti ICIs predstavuje imunohistochemická analýza expresie ligandu 1 programovanej bunkovej smrti (PD-L1), hodnotenie tumor infiltrujúcich lymfocytov (TIL) v nádorovom mikroprostredí (tumor microenvironment – TME) a vyšetrenie nádorovej mutačnej nálože (tumor mutational burden – TMB). Nové biomarkery súvisiace s aktiváciou signálnej dráhy transformačného rastového faktora beta, s receptorom 1 domény diskoidínu a s trombospondínom 1, ako aj mnohé ďalšie celulárne a molekulárne faktory prítomné v TME, predstavujú potenciálne prediktory účinnosti ICIs využiteľné v budúcnosti. ***Cieľ:*** V predkladanom prehľadovom článku sumarizujeme súčasné poznatky o regulácii PD-L1 a o prediktívnej hodnote TIL a s nimi súvisiacimi celulárnymi a molekulárnymi komponentmi prítomnými v systéme TME v TNBC. Tiež sa venujeme TMB a novým biomarkerom s potenciálnou úlohou v predikcii účinnosti ICI a pokúsime sa načrtnúť nové terapeutické stratégie.

Kľúčové slová

triple-negatívny karcinóm prsníka – imunoterapia – ligand 1 programovanej bunkovej smrti – tumor infiltrujúce lymfocyty – nádorová mutačná nálož

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Introduction

Triple-negative breast cancer (TNBC) is the appellation used to describe carcinomas of the breast which are negative for estrogen receptors (ER) as well as progesterone receptors, and which lack overexpression of human epidermal growth factor receptor 2 (HER2) [1]. About 10–20% of breast carcinomas meet the diagnostic criteria of TNBC [2]. Histologically, these relatively common tumors represent high-grade malignancies, which behave clinically in an aggressive fashion [3]. Distant metastases occur early in the clinical setting and the disease-specific survival of patients with TNBC is poor when compared to other breast carcinoma subtypes [4,5]. The negative hormone receptor status renders tumor cells of TNBC irresponsive to selective ER modulation therapy [6,7], and the lack of *HER2* amplification makes clinical success and efficacy of treatment with trastuzumab [8] or other types of targeted therapy [9] highly unlikely. Therefore, standard chemotherapy regimens remain the mainstay in handling TNBC [10–12], and effective treatment modalities are urgently needed to meliorate the dire clinical outcome of oncological patients having this type of breast cancer [13].

The very important role of immune checkpoints in regulating immune responses engaged in the cancer-elimination process has been corroborated in various human malignancies [14]. Programmed cell death protein-1 (PD-1) represents the immune checkpoint which delivers the most robust immunosuppressing signals [15]. In previously activated T-cell, PD-1 causes exhaustion [16] or anergy [17]. Cancer cells are capable to activate PD-1 on tumor-antigen specific T-cells by aberrantly expressing on their surface programmed death-ligand 1 (PD-L1) [18]. Immune checkpoint inhibitors (ICIs) are novel immunotherapy agents, which cause the disruption of the interaction between PD-1 and PD-L1 [18]. This reinvigorates tumor-specific cytotoxic CD8+ T cells and ultimately leads to cancer cell elimination [19]. Over the past several years, treatment with ICIs has repeatedly proven to be superior to conventional chemotherapeutical approaches by granting significant survival benefit in oncological patients having various types of cancer, including malignant melanoma [20], advanced non-small cell lung carcinoma [21,22], head and neck cancer [23], urothelial cancer [24], and Hodgkin lymphoma [25]. Analysis of PD-L1 expression on the surface of tumor and/or immune cells using immunohistochemical methods is the most widely used marker whose value in predicting efficacy of ICIs has been corroborated in clinical studies in various cancer types [26]. In TNBC, indication of treatment with ICIs is based mostly on analytical results obtained from evaluation of PD-L1 expression on tumor infiltrating immune cells. These include lymphocytes, macrophages, dendritic cells, as well as granulocytes. If at least 1% of the tumor area contains PD-L1 positive tumor infiltrating immune cells, treatment with atezolizumab (an ICIs blocking PD-L1) is indicated [27].

Immunotherapy approaches in most breast cancer forms have not been well established yet, because of the reported low immunogenicity of these tumors [28]. However, the TNBC subtype seems to stand out as an exception, because several immunological attributes with proven predictive value have been identified in this form of breast cancer [29]. First, TNBC have a reported higher level of PD-L1 expression present mostly on tumor infiltrating immune cells [30]. Secondly, compared to other breast cancer subtypes, tumor infiltrating lymphocytes (TILs) occur in TNBC more often and in higher number [31]. The presence on TILs has been linked to survival benefit from treatment with ICIs in TNBC patients [32]. Finally, these malignancies are referred as having a high tumor mutation burden (TMB), which is related to the large amount of non-synonymous somatic mutations per coding area present in their genome. This ultimately leads to neoantigen production and higher immunogenicity of TNBC [33]. Furthermore, various components within the tumor microenvironment (TME) play, presumably, additional important roles in regulating antitumor immune responses and have the potential of being harnessed to improve immunotherapy in the future [34].

In this review, we summarize the current knowledge of mechanisms regulating PD-L1 expression in TNBC. We also discuss the predictive role of TILs and other known associated cellular and molecular components present within the TME of TNBC. Furthermore, TMB and emerging biomarkers with potential of predicting efficacy of ICI are discussed. New therapeutic strategies will be outlined.

The predictive value of PD-L1 expression in TNBC

Results published from early clinical studies have shown durable response with the monoclonal antibody atezolizumab as monotherapy or in combination with standard chemotherapy for advanced TNBC, especially in patients having PD-L1 positive breast cancer [35,36]. In patients having advanced TNBC, the era of immunotherapy has been launched at the end of year 2018, when results of the phase III clinical trial IMpassion130 were published [37]. In their study, Schmidt et al [37] demonstrated a significant clinical benefit in patients with metastatic or locally advanced TNBC treated with a combination of atezolizumab and nanoparticle albumin-bound (nab) paclitaxel. Patients in the nab-paclitaxel – atezolizumab group showed a statistically significant improvement in the median progression free survival (PFS) and median overall survival (OS). This improvement was especially pronounced if the TNBC stained positive for PD-L1 [37]. Contrary to these encouraging results, the IMpassion131 study did not show a significant survival benefit in the subgroup of patients receiving the combination of atezolizumab with paclitaxel [38]. The reasons for these disappointing results remain to be determined.

Without proper selection of patients with metastatic or locally advanced breast cancer, meaningful clinical responses are achieved in only about 10% of TNBC patients treated with a combination of ICIs and standard chemotherapy [39]. The TME of TNBC disposes with numerous cellular and molecular factors

with possible impact on the quality of antitumor immune responses [36], and PD-L1 expression seems to be just one factor that needs to be taken in account when ICIs treatment is considered [41]. According to published data, the overall PD-L1 positivity in the different subtypes of breast cancer varies within the range of 20–34% [42–44]. However, PD-L1 immunohistochemistry seem to represent an imperfect predictive biomarker for some patients., as is suggested by published results obtained from the study of other cancer types [45,46]. Indeed, a relatively high proportion of oncological patients having PD-L1 positive malignancies belong to the non-responder category, while many achieve proven clinical benefit and radiological confirmed response despite having PD-L1 negative tumors [47].

Questions related to the interchangeability of PD-L1 immunohistochemistry assays arise [48]. Currently, in patients with metastatic or locally advanced TNBC, the Food and Drug Administration (FDA) approved the Ventana PD-L1 (SP142) immunohistochemical assay as a companion diagnostic tool for indication of treatment with atezolizumab – nab-paclitaxel [49]. Approval was based on published results from the IMpassion130 trial as well as other clinical studies using the SP142 platform [39,50,51]. The anti-PD-1 agent pembrolizumab, which utilizes the 22C3 assay as a companion diagnostic tool, was approved for early stage TNCB [52]. Inconsistencies in results exist regarding immunohistochemical assays for other types of ICIs, which may be a result of differences in the staining abilities of these diagnostic assays and variability in the chosen cut-offs defining the status of PD-L1 expression [53].

Even if harmonization of the various PD-L1 assays in TNBC would be achieved, another problem regarding the reliability of results obtained from PD-L1 immunohistochemistry is related to the heterogeneous distribution of this predictive marker within the tumor [54]. Heterogeneity of PD-L1 is a well-described phenomenon in various cancer types in human oncology [54]. From a practical point of view, irregularities of PD-L1 protein distribution can lead to misinterpretation of the status of PD-L1 expression in tumors when only biopsies are used for the purpose of immunohistochemical analysis [55]: poor concordance has been found when comparing results of PD-L1 status obtained from biopsy samples with those results determined by resection specimen evaluation [55]. Misclassification of the PD-L1 status may be one explanation of the phenomenon, when oncological patients having "PD-L1-negative" tumors gained survival benefit and demonstrated obvious efficacy after ICIs therapy was applied in the second line [47]. The reason may be that a different area in the given tumor expressed PD-L1 and was therefore sensitive to immunotherapy [55]. Furthermore, PD-L1 expression has been found to be associated with higher-grade histological growth patterns in malignancies characterized by morphological heterogeneity [56]. Heterogeneity of PD-L1 as well as other biomarkers has been shown to be present and is highly pronounced also in tumor tissue of TNBC, which may represent an analytical problem that needs to be addressed [57]. A possible way how to optimize PD-L1 immunohistochemistry in TNBC is to increase the number on core-cut biopsies in situations where resection specimens, which would enable a more comprehensive evaluation of the PD-L1 status, cannot be obtained [58]. Discrepancies in the reported PD-L1 expression status have also been observed between metastatic lesions and their primaries, which raises questions about the need of combining results from both localizations to be able to identify patients suitable for immunotherapeutic approaches more accurately [59].

Furthermore, the level of PD-L1 expression in TNBC has been found to dynamically change in time [60] and is markedly influenced by the effects of common treatment modalities including standard chemotherapy and radiotherapy [61,62]. Because PD-L1 expression is so closely related to numerous external factors, post-treatment evaluation of PD-L1 status could be a reasonable approach in the future [61].

Another matter of dispute is whether the staining of PD-L1 protein, determined immunohistochemically, should be analyzed on tumor cells and/or lymphocytes [63]. The evaluation of PD-L1 expression on the surface of tumor cells has been proven to be of high predictive relevance in several human malignancies [63], with the most valid clinical results in non-small cell lung carcinoma patients [63,64]. In TNBC, PD-L1 expression is restricted mostly to immune cells with a reported overall positivity of 34% [43]. In the IMpassion130 trial, overall positivity of PD-L1 in at least 1% of immune cells was identified as the cut-off defining the patient population most suitable for first line treatment with atezolizumab – nab-paclitaxel combination [39]. Of note, most TNBC cases infiltrated by PD-L1 positive immune cells also contained tumor cells which stained positive for PD-L1 [39]. Subgroup analysis has shown a positive correlation between the number of cytotoxic CD8+ T cells and PD-L1 positivity in immune cells, and both factors were concomitantly associated with better PFS and OS [39]. These results underline the important functional interconnections between TILs and PD-L1 expression in the TME of TNBC [65].

The predictive value of TILs in TNBC

The TME of TNBC contains a plethora of immune cells which can be broadly divided into two categories: immune cell with proinflammatory properties include T helper type 1 cells, cytotoxic CD8+ T cells, M1 macrophages, dendritic cells, and natural killer cells [66]. On the other hand, a TME with immunosuppressive properties is dominated by M2 macrophages, myeloid-derived suppressor cells, and regulatory T cells, the latter being the major culprits responsible for perturbations in antitumor immunity [66]. TILs is the umbrella term which describes mononuclear immune cells localized in the close vicinity of tumor tissue or directly infiltrating intratumorally [67]. TILs have been described in many types of human cancer [68,69]. The presence of TILs within the TME, which can be determined using simple diagnostic slides stained with hematoxylin and eosin, has been suggested as an

indicator of preexisting anticancer immunity [70]. Regarding the spectrum of breast malignancies, the TNBC subtype has been found to be most often associated with a dense infiltrate of TILs [71].

The presence of TILs gained much attention over the past several years as potential biomarkers in TNBC, which are able to estimate prognosis of patients and predict the efficacy of treatment with ICIs [72]. A significant linear relationship between increased levels of TILs and recurrence-free survival has been observed in TNBC patients [73]. Luen et al [74] corroborated the clinical relevance of TILs assessment as being an accurate and easily reproducible method to properly estimate the prognosis of TNBC patients in the metastatic setting. Regarding the feasibility of using TILs to predict treatment outcome, evidence derived from analysis of TNBC cases points to a strong positive correlation between increased levels of TILs and pathological complete responses (pCR) to neoadjuvant chemotherapy [74]. Furthermore, because the presence of TILs serves as an indicator of higher tumor immunogenicity [68], their potential to predict clinical outcome of immunotherapy in TNBC has been tested: in TNBC, the presence of stromal TILs positively correlated with pCR in patients treated with neoadjuvant chemotherapy and pembrolizumab [52]. Gonzalez-Ericsson et al [75] came to the conclusion that PD-L1 expression and TILs represent a continuous spectrum of immunological processes within the TME of breast cancer, and they suggest that the most effective way how to identify patients most suitable for treatment with ICIs is to assess PD-L1 and TILs as a composite biomarker [75]. Collectively, these results suggest that the TME of TNBC disposes with a robust repertoire of tumor-specific T cells whose cancer-eliminating activity can be bolstered by ICIs administration [66].

The association between the presence of TILs and PD-L1 expression within TME has been studied and corroborated in several other forms of cancer [76]. Many proinflammatory cytokines and related mediators are elaborated by activated T cells, which act synergistically as extrinsic signals eventually leading to upregulation of PD-L1 in cancer cells as well as in TILs [77]. Out of these inflammatory products, interferon γ (IFN-γ) is responsible for the most robust increase in PD-L1 positivity in the various cellular components present in the TME [77]. It has been shown that the effectiveness of cancer-eliminating immune responses, interconnected with the regulation of PD-L1 expression in the TME, is affected by the qualitative proportion of various T cell subpopulations [78]: forkhead box protein 3 (FOXP3) positive regulatory T cells play a pivotal role in the development of a TME with immunosuppressive properties especially through their ability to substantially increase PD-L1 expression [78]. Oshi et al [67] have demonstrated enhanced cancer-directed cytolytic activity of cytotoxic CD8+ T cells when concomitant increase in the number of CD4+ memory T cell was present in the TME of TNBC. They propose that the application of CD8+ T cell scores, which correlate strongly with the expression of *CD8* genes as well as a with *IFNαγ* and *IFNγ* gene signatures, can be used for the purpose of predicting clinical outcomes of ICIs treatment in TNBC patients [67].

Besides aberrant PD-L1 expression, cancer cells are known to have various other mechanisms which hamper effective antitumor immune responses [77]. One of the most important cell type present in the tumor stroma are cancer-associated fibroblasts (CAF), which elaborate and remodel the constituents of the extracellular matrix [79]. It has been demonstrated that activation of the transforming growth factor β (TGF-β) signaling pathway in CAF leads to increase in collagen fiber production by these cells [80]. Deposition of dense collagen fibers within the stromal compartment of TME causes exclusion of TILs and is responsible for ineffective cancer-eliminating immune function of cytotoxic CD+ T cells [80]. Furthermore, activation of the TGF-β signaling pathway was associated with higher levels of PD-L1 expression in tumor cells [81]. Preclinical studies testing concomitant blockade of PD-L1 and the TGF-β axis are ongoing, and the results have been promising so far [81]. Nevertheless, the role of TGF-β signaling in TNBC has not been extensively studied yet, and prospective studies related to this topic are recommended.

Exclusion of TILs and disabling them from conducting their cancer-eliminating function is associated with poor clinical outcomes of patients having TNBC [72]. Besides the activation of the TGF-β axis [80], there are currently only few known mechanisms related to stroma-remodeling with possible therapeutic implications [82]. The discoidin domain receptor 1 (DDR1) is a major factor which mediates interaction between epithelial cells and stromal components in the human mammary gland during the process of ductal structure development [82]. Sun et al [83] have described an important molecular regulatory network mediated through DDR1, which contributes to immune exclusion in TNBC: DDR1 acts as a collagen receptor and has tyrosine-kinase activity [83]. Activation of DDR1 causes alignment of collagen fibers in the extracellular matrix, creating a dense meshwork that serves as a mechanical barrier within the TME of TNBC which TILs are not able to cross [83]. Moreover, Sun et al [83] also demonstrated that reversion of immune exclusion can be achieved by ablation of DDR1 in mouse models of TBNC. Consistent with this, in the TME of human TNBC, abundance of cancer eliminating cytotoxic CD8+ T cells was inversely correlated with the expression of DDR1 [83]. Neutralizing antibodies directed against the extracellular domain of DDR1 caused disruption of collagen fiber alignment, mitigation of immune exclusion, and inhibition of tumor growth in hosts with a functioning immune system [83]. Conclusively, therapeutic strategies causing reconfiguration of the extracellular matrix within the TME of TNBC can cause reversion of immune exclusion and possibly potentiate the efficacy of ICIs [83].

Yet another recently described mechanism of immune exclusion described in TNBC is related to expression of thrombospondin 1 (TSP1) [84]. Expression of TSP1 has pleiotropic effects in the TME of TNBC including activation of the epithelial to mesenchymal transition in cancer

cells, increase in the metastatic potential of tumors, and activation of the TGF-β axis [84]. Marcheteau et al [84] have demonstrated that TSP1 expression is inversely correlated with the presence of cytotoxic CD8+ T cells within the TME of TNBC. Knockdown of TSP1 caused a significant increase in the number of TILs in mouse models having TNBC and functioning antitumor immunity [84]. Inhibition of TSP1 could be a possible therapeutic approach in the future [84].

The predictive value of TMB in TNBC

Although PD-L1 immunohistochemistry and the assessment of TILs in the TME of TNBC are currently well-established factors, which are evaluated when immunotherapeutic approaches are considered, clinical outcome of ICIs treatment remains in many breast cancer patients unpredictable [29]. Because of this, there is a need for enrichment with predictive factors [29].

The general immunological principles of effective cancer-eliminating immune responses require the recognition of cancer cells foreign [17]. Therefore, the mutational status of malignant transformed cells, which leads to neoantigen production, has gained attention as a potential predictive marker of ICIs efficacy [85]. The FDA granted approval for pembrolizumab for any type of unresectable and metastatic cancer with microsatellite instability (MSI+) or mismatch-repair deficiency (MMR–) [86]. MSI+ and MMR– are known molecular states associated with progressive acquisition of somatic mutations [33]. TNBC is known to harbor a higher number of somatic mutations than other subtypes of breast cancer [85]. Nevertheless, only approximately 2% of TNBC cases meet the criteria required to be classified as MMR– tumors or MSI+ cancer, which suggest alternative ways how tumor cells of TNBC acquire mutations [86].

Another measurement of genetic alterations present in the genome of cancer cells is TMB, which quantifies more generally the mutational status of a given malignant tumor [86,87]. TMB is defined as the average number of somatic mutations per megabase (mut/MB) in the genome of tumor cells and is determined using whole exone sequencing [88]. If the tumor harbors 10 ≥ mut/MB, it is designated as having high TMB [89]. Positive correlations between effectiveness of ICIs and high TMB have been demonstrated in several types of human malignancies [90]. Compared to PD-L1 expression and TILs evaluation, which are examples of direct measurements of the immune activity, TMB reflects indirectly the number of neoantigens, which are presented through the major histocompatibility complex to cytotoxic CD8+ T cells [85]. As such, a higher level of TMB in TNBC tends to correlate with other immune parameters of response to ICIs [89]. Metastatic TNBC tend to have a higher TMB compared to their primaries, which is possibly a result of the tendency of more genetically unstable cancer cells to acquire a higher number of mutations granting them survival benefits and more invasiveness [90]. Although some preliminary results from recent clinical trials suggest that TMB could have some value in predicting pCR of early-stage TNBC treated with ICIs [93], results are significantly more convincing in the metastatic setting [94]. The KEYNOTE-119 clinical trial showed a positive correlation between high TMB and clinical benefit from pembrolizumab monotherapy in patients with TNBC [94]. Analogically to the afore-mentioned immunotherapy related biomarkers, TMB should be viewed in the context of other factors present in TME.

Conclusions

Currently, the most clinically valid markers used to predict efficacy of immunotherapy in patients with advanced TNBC remain the immunohistochemical analysis of PD-L1 protein expression, the assessment of TILs present within the TME, and the evaluation of TMB of the malignant cells. Because the effectiveness of ICIs remains unpredictable in a substantial proportion of TNBC patients, there is an urgent need for enrichment in effective biomarkers. Emerging biomarkers with potential to be utilized in the future are related to activation of the TGFβ signaling pathway, DDR1, and thrombospondin-1 as well as to other cellular and molecular factor present within the TME. A deeper understanding of the intricating molecular mechanisms which drive TNBC growth could eventually lead to inception of new immunotherapeutic modalities of this highly malignant disease.

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