JBUON 2019; 24(3): 883-888
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

REVIEW ARTICLE

PD-1 and PD-L1 as immunotherapy targets and biomarkers in non-small cell lung cancer

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Summary

The integration of immunotherapeutic agents in the treatment of non-small cell lung cancer (NSCLC) has revolutionized the approach of the prevalent type of lung cancer. Although PD-1 and its ligands (PD-L1 and PD-L2) are stimulating molecules of the immune-checkpoint pathway, with primary function to limit inflammatory response and autoimmunity, tumor cells have found a way to exploit these molecules by obtaining the opportunity to respond with PD-L1 expression in cytokine signals and thus to evade immune surveillance. Several immunotherapeutic agents targeting these molecules have already been tested and show quick and remarkable responses and survival prolongation in about 14-20% of chemo-resistant patients in NSCLC, resulting to FDA approval of some PD-1 inhibitors (pebrolizumab, nivolumab), even for first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression (pebrolizumab). Regarding to the prognostic value of PD-L1 and PD-1 expression as biomarkers in NSCLC, the results still remain contradictory. However, the elevated expression of PD-L1 has been correlated with higher efficacy of the various immunotherapeutic agents, implying a high predictive value of this biomarker, even if the truth about specificity and sensitivity of the aforementioned molecules is generally more complicated.

Key words: *PD-1, PD-L1, immunotherapy, immune-oncology, biomarkers, NSCLC*

Introduction

Every year, 1.59 million people are diagnosed and 1.81million are dying of lung cancer, making the disease the first cause of cancer-related deaths worldwide [1]. With tobacco use being its most important risk factor, non-small cell lung cancer (NSCLC) is the most common type of lung cancer accounting for about 80% of all cases, and is diagnosed more frequently in patients over 65-year-old [2-5]. Despite the advances in technology and new diagnostic techniques, NSCLC is still diagnosed mostly in metastatic stages, especially in peripheral located disease, due to lack of early symptoms [3]. A standardized therapeutic protocol for early-stage neoplasms contains surgical removal, while cytotoxic platinum-based chemotherapy remains the main approach for advanced disease, excluding the cases with specific genomic aberrations, where certain targeted molecular therapy is used as first-line treatment [4]. The problem was always identified in second-line treatment, were no single agent managed to remarkably prolong the overall survival (OS), despite the improvement in progres-

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Received: 29/08/2018; Accepted: 02/10/2018

sion-free survival (PFS) [5]. The solution was given by the recent development of immunotherapy that represents a radical shift to cancer treatment [6].

Lung cancer was thought to be poorly immunogenic, because all the efforts to modulate immune system, mostly represented with vaccines, turned to be totally ineffective due to inappropriate immune activation. Even the TG4010 vaccine, that presented some promising results, is still under investigation [7,8]. Nevertheless, the molecular understanding of the inhibitory checkpoint pathways has revolutionized the immunotherapeutic approaches of lung cancer. There are two checkpoint molecules that their blocking has shown an early remarkable clinical success [9]. The first molecule is the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4 - B7.1) that inhibits T-cell activation from dendritic cells in the lymph nodes and the second one is the PD-1(Programmed death protein 1) that inactivates T-cells when binding to its ligands [10]. Tumor cells exploit these two inhibition paths in order to escape from immune surveillance. In fact, by blocking these molecules, the host immune system is set in target and not the intratumoral signaling defect and here is identified the success of this approach in a wide variety of tumors, comparatively with treatments that target specific oncogenic defects [6,11].

The role of Programmed Death protein 1 (PD-1) and its ligands

PD-1 and its ligands (PD-L1 and PD-L2) are stimulating molecules of the immune checkpoint pathway with primary function to dampen the effector phase of activated T-cells in peripheral tissues and to limit inflammatory response and autoimmunity [12]. PD-1 is a transmembrane protein, expressed on various blood cell types such as B cells, T cells, NK cells, dendritic cells and Treg [13,14] and is upregulated in the effector phase of the immune response [14]. PD-L1 is expressed on various cell types, such as muscle, pancreatic islet cells, placenta, mesenchymal stem cells, T cells, B cells, dendritic cells, macrophages and mast cells [15]. On the other hand, PD-L2 expression is restricted in more specific cells, mostly on macrophages and dendritic cells, indicating its limited role in T-cell polarization or priming, in comparison with PD-L1 which contributes with its expanded role in protecting the tissues from autoimmunity or overactivated inflammatory response [6,16]. During the immune response, the cataclysmic production of INF-γ, leads to up-regulation of PD-L1, forming a protective immune layer on the expressing cells. The reaction and binding of PD-1 to PD-L1, inhibits kinase signaling paths and thus carries suppressive messages for T-cells, downregulating them by causing their exhaustion or apoptosis and preventing the autoimmune attack [14,17].

Emerging research has identified PD-1 as an immunosuppressive checkpoint pathway that tumor cells may exploit to evade immune surveillance [14]. Deletion or mutation of certain genes (i.e. PTEN -phosphatase and tensin homolog) give to cancer cells the opportunity to respond with PD-L1 expression, in many inflammation signals induced by cytokines, with INF-γ playing the most important inducing role [18]. PD-L1 expression has been already detected in many cancer types, such as melanoma, gastric and renal cancer, glioblastoma, NSCLC, various leukemias and so on [19,20]. The molecule can be transmembrane, forming a molecular "immune shield", or can be found in the cytoplasm in a number of cells that varies, depending on the histology type and the organ [21].

NSCLC immunotherapy with antibodies against PD-1 and PD-L1

The exploitation of the inhibitory checkpoint molecules by the cancers cells gave birth to the idea of antibody immunotherapy targeting these molecules. By inhibiting PD-1 and PD-L1 interaction, not only the activity of the immune system is intensified, but also the tumor "immune shield" is disabled, resulting to a boosted anti-tumor effect [22]. In fact, targeting PD-1 or PD-L1 belongs to the same therapeutic approach, with slightly different effects [24]. Several trials conclude that PD-1 (pebrolizumab, nivolumab) as well as PD-L1 inhibitors (atezolizumab, durvalumab, avelumab) have shown incredibly optimistic results in many cancer types [23] (Figure 1). Especially for NSCLC, the numerous trials, that have already been conducted, show quick and remarkable responses in about 14-20% of chemoresistant patients and survival prolongation [24]. Indeed, FDA has already approved the use of nivolumab, atezolizumab and pembrolizumab as second-line therapy for advanced NSCLC with disease progression on or after platinum-containing chemotherapy [25]. The improvement in OS seems to be independent of PD-L1expression when using nivolumab or atezolizumab, but the clinical efficacy of pembrolizumab is highly associated with the PD-L1 positivity [26]. Today, pembrolizumab has been approved for treatment of patients with metastatic NSCLC whose tumors express PD-L1 (Tumor Proportion Score-TPS ≥1%) but also for first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression (TPS) ≥50%) [27].

The prognostic value of PD-L1 and PD-1 expression

Overexpression of PD-L1 is mentioned as negative prognostic factor in many tumor histologies [6]. Although many studies investigated the value of PD-L1 as prognostic biomarker in NSCLC, the results still remain contradictory. While in some cases the increased expression of PD-L1 appears to be predictive for an extended OS [28], in some others is a factor unrelated to prognosis [29], but in most studies high PD-L1 expression is presented as a negative prognostic marker [28,30]. In fact, in one study it was noted that poor tumor differentiation, which is directly associated with increased PD-L1 expression, was the cause of poor OS and thus poor prognosis in NSCLC [20,31]. According to a meta-analysis, high PD-L1 expression is compatible with worse survival only in non-treated patients, a fact that is reversed when the patients get treated further postoperatively with radiation or chemotherapy, and then no difference in survival rate is observed [32]. Indeed, the recurrence rate in patients with high expression of PD-L1 is doubled in stage I NSCLC, probably because they don't receive any adjuvant therapy according to the current protocols [33]. Consequently, the prognostic implication of PD-L1 expression still remains uncertain.

Regarding the relevance of PD-1 expression for NSCLC prognosis, only a few is known [34]. It has been observed that excessive expression of PD-1 by tumor infiltrating T-lymphocytes (TILs) is combined with exhausted phenotype, which is translated into reduced cytokine secretion and low proliferation potential [35].

The predictive value of PD-L1 expression

It could be suspended that PD-L1 expression is a predictive biomarker for anti-PD-L1 immunotherapy on the basis of better response to PD-L1 blockades in NSCLC [36]. Even if this conclusion is the most obvious, the truth is more profound. In numerous studies, the elevated expression of PD-L1 has been correlated with higher efficacy of the various immunotherapeutic agents, that redounds to a superior OS and PFS [36-38]. However, PD-L1 overexpression doesn't mean clinical efficacy automatically. For example, it seems that the clinical response to nivolumab is not related with the PD-L1 level in squamous cell NSCLC, maybe because the response is mediated from the mutation load and mutational signature or just because squamous cell carcinoma is a high immune-infiltrating tumor [39]. Finally, maybe PDL1 expression did not correlate with response because archival tissue was often used for PD-L1 determination [40]. Impressive though is the fact that beyond the misty relation between high PD-L1 expression and improved OS, there is another complicated connection between the lack of PD-L1 expression and the clinical efficacy of anti-PD-L1 immunotherapy. In fact, the confusion is provoked from the remarkable number of negative responders that reaches the percentage

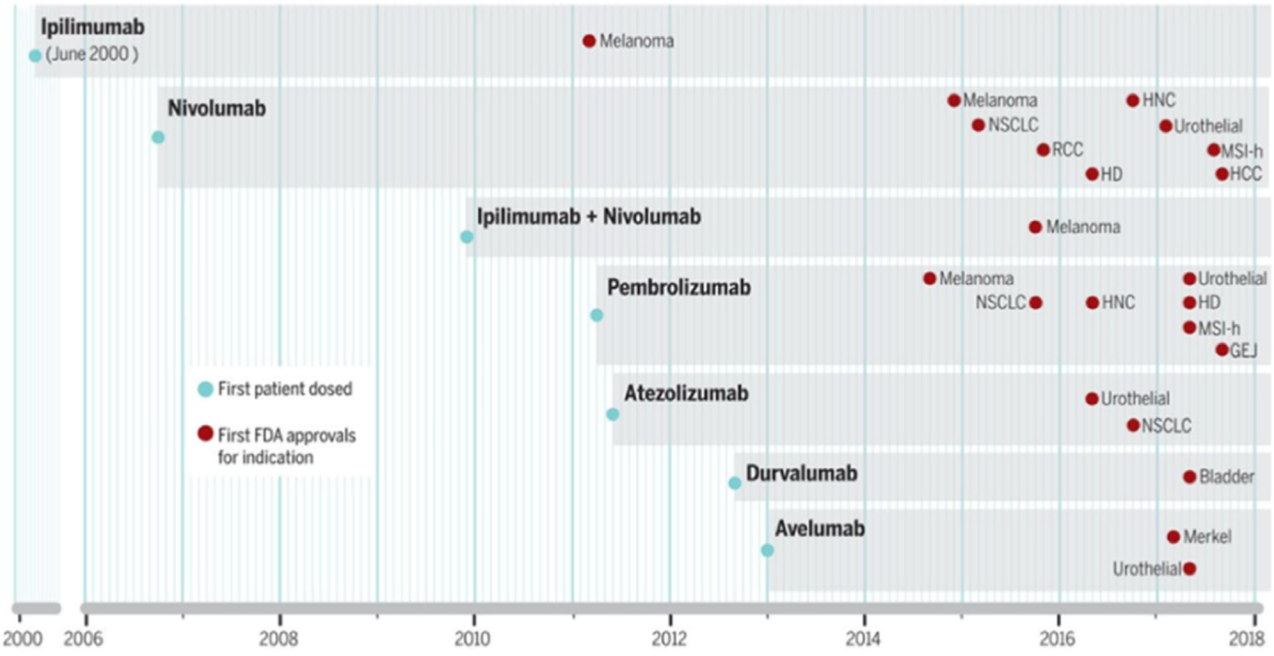

Figure 1. Timing of clinical development of anti–CTLA-4, anti–PD-1, and anti–PD-L1 antibodies, from first administration to humans to FDA approval [25].

of 8-20% [6]. These patients respond exceptionally to the anti-PD-L1 immunotherapy, even if the PD-L1 is less than 1% [41].

The explanation for the diversity in the effectiveness of the anti-PD-L1 molecules is complicated and multifactorial. First of all, the PD-L1 expression is dynamic in space and time and heterogeneous, which means that differs depending to the histological type, and dynamic, because it changes over time [11]. Some researchers claim that the biopsy measuring the percentage of PD-L1 is only a snapshot of the tumor microenvironment at the certain moment, directly dependent from the cytokines expression like IFN-γ, while PD-L1 expression is affected and induced as much from the upregulating messages of the TILs, as from the genetic changes in the tumor [42]. Moreover, PD-L1 expression is changing after chemotherapy and radiation, and sometimes is different between the primary and the metastatic lesions [43]. In fact, one more explanation for the diversity in clinical response to this kind of therapy is the lack of standardization and the various cut-off points in PD-L1 expression [36,44,45]. Furthermore, it is not clarified if the staining is referring to the tumor cells, the TILs, or the whole tissue section and if PD-L1 expression on tumor cells (TC) and tumor-infiltrating immune cells (IC) is an additional prognostic factor.

All these said it is assumed that PD-L1 is not the only predictive parameter in NSCLC. More specific and more sensitive biomarkers should be found, that they don't exclude potential responders.

Conclusion

It is obvious that PD-1/PD-L1 checkpoint blockade has revolutionized the second-line treatment of NSCLC patients. Although considerations do not stop rising, mainly regarding the detection of biomarkers that will accurately identify the patients that will benefit from the treatment and those who won't. PD-L1 molecule may have the potential to be one such biomarker, but the most important thing is the definition of the ideal PD-L1 TPS expression that is compatible with clinical benefit. Moreover, it has to be scrutinized if the degree of staining in TILs, tumor cells or the whole tissue separately is combined in differences in OS or the PFS.

Conflict of interests

The authors declare no conflict of interests.

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