Review

PD-L1 and PD-1 screening and therapy in non small cell lung carcinoma

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Abstract

Lung cancer is still the leading cause of death among all malignancies worldwide. The development of targeted therapies against driver mutations such as EGFR, ALK1, ROS1 and BRAF have led to a significant improvement in patient progression free survival and to a benefit in quality of life of patients suffering from advanced and metastasized non-small cell lung cancer. But since these genetic aberrations are found only in a small subset of lung cancer more globally directed therapeutic approaches are needed to address the therapeutic dilemma of this highly diverse disease. For a long time, it is known that lung cancer is a so called immunogenic disease, i. e. it often evokes a host immune response. Likewise, lung cancers are also developing mechanisms to escape these anti-cancerous immune reactions. One immunogenic axis is that of PD1 and PD-L1. In investigation of this activation-deactivation chain involving lymphocytes, tumor cells but also stromal fibroblasts and macrophages new humanized antibodies have been developed and approved for the treatment of non-small cell lung cancer (NSCLC). Clinical trials have shown effectiveness of these agents, but a valid and reproducible predictive marker has not been found so far. Here we review the current literature on the PD1/PD-L1 axis in NSCLC and its biological function on histological subtype pf NSCLC. Together with meta-analytic data performed for this review and results from our own investigations we also give a comprehensive review on current developments considering predictive testing and therapeutic options.

Keywords: non small cell lung cancer, PD1, PD-L1, immune checkpoint therapy

Strategies for immunotherapies through the centuries

First therapeutic approaches in the field of immunotherapy date back to 1893, when a tumor cell loss of MHC class 1 molecules and many other mechanisms of cancer progression were later described as part of the hallmarks of cancer [4]. Stimulating the immune system in an unspecific manner through cytokines like interferon-alpha and interleukin-2 has been a major pillar of tumor therapy for many years. The first specific therapy targeting mechanism of immune system anergy was a monoclonal antibody called Ipilimumab which binds CTLA-4, a conveyor of central and peripheral tolerance [5]. Lately, blocking antibodies aiming at the Programmed Death receptor 1 (PD-1) , which was detected in T-cell hybridomas undergoing programmed cell death [6], and its ligand (PD-L1) has seen accelerated development and approval across many entities.British physician successfully treated a young man who had an inoperable tumor by repeatedly inoculating a bacterial lysate to induce local tissue inflammation [1]. However, and as one can imagine, these early attempts to harness the power of the immune system lacked clinical success and had considerable adverse effects. Later, Paul Ehrlich introduced the concept of "immune surveillance" hypothesizing that the immune system specifically detects and suppresses cancer cells and failure of this process would

result in the manifestation of disease [2]. Later on, the discovery of tumor antigen-specific immune cells and vaccine induced tumor immunity confirmed this theory and ignited further research into the field [3]. Antigen-specific T-cell anergy,

PD-1 and PD-L1

PD-1 is a trans-membrane receptor weighing 55kDa, composed of 288 amino acids and occurs on activated T-cells, B-cells, natural killer cells, monocytes and dendritic cells [7]. Extracellularly, there is an immunoglobulin-superfamily binding site which structurally resembles that of the related coinhibitory molecule CTLA-4. Intracellularly the receptor has two domains responsible for signal transduction through a chain of phosphorylation and dephosphorylation which results in inhibition of the T-cell receptor signal [8, 9]. This action results in a diminished IL-2 production and inhibition of other cellular pathways like PI3K and AKT [10, 11]. These as well as other cellular pathways lead to cell anergy, is associated with cell death and is thought to display inactivated cells after overstimulation [12].

PD-L1 is one of the known ligands of PD-1. The molecular structure of the PD-1 - PD-L1 binding complex is displayed in figure 1 [13].
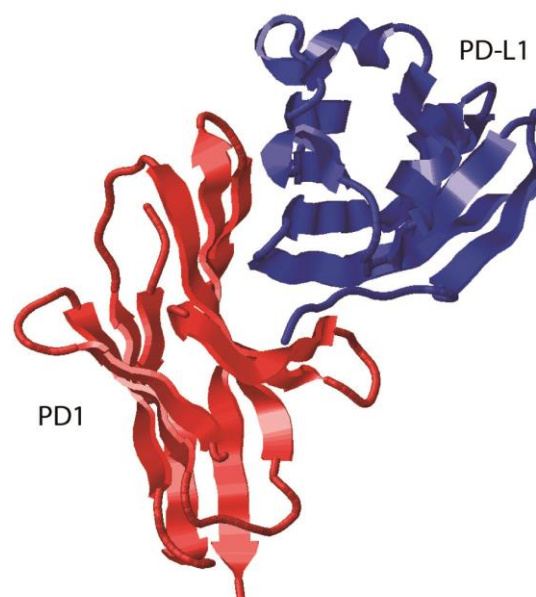

Figure 1:

Molecular structure of the binding site of the PD1-PD-L1 complex. Modeled with RasMol 2.7.5 [67] upon data of published structure data [13]

As such it is partially constitutively and variably expressed in different tissues and cells. It can be expressed in both hematological cells such as B- and T-cells, myeloid cells and macrophages as well as non-hematological cells such as epithelium, endothelium and cells derived from the endoderm such as hepatocytes and pancreatic islet cells [14]. In part its function can be understood by the pattern of expression for example in syncytiotrophoblast epithelium cells as a way to elicit peripheral tolerance [15]. PD-L1 also seems to have importance in establishing self-tolerance centrally as it is expressed in thymocytes during the phase of negative selection of T-cells [16]. The function can also be appreciated by looking at a range of autoimmune phenomena and infectious diseases. For example, the high level of tolerance for heart and liver transplants in non MHC-compliant recipients may be partially due to the fact of a high constitutive level of PD-L1 expression in these tissues [17].

Mediation of self-tolerance by a PD-1 dependent mechanism has also been revealed in mouse models for dilatated cardiomyopathy and type 1 diabetes [18, 19]. Many studies have shown that the PD-L1 - PD-1 axis can have profound impact on the course of infections. The chronic infection of the gastral mucosa by Helicobacter pylori leads to an upregulation of PD-L1 in the epithelial cells, dampening the immune response and potentially facilitating bacterial colonization [20]. Similarly, in PD-1 knockout mice bacterial infections were cleared significantly quicker than in healthy, PD-1 wild-type mice [21]. The role of PD-1 has also been studied in viral infections. In viral hepatitis, the expression of PD-1 and its ligand is correlated with a decreased reduction of the viral load [22]. Comparable results have been published for LCMV. Here it was even possible to elicit a stimulation of virus-specific T-cells by PD-1 blockade and thereby achieve a reduction of the viral load [23]. This proved that the inhibition of T-cells through PD-1 can be reversible, which other studies of viral infections showed, too [24].

The best known inductor of PD-L1 expression in all tissues and cell types is IFN-γ (Interferon- γ) [7]. Additionally, the expression of PD-L1 on lymphatic cells was shown to be upregulated by a range of cytokines and molecules including CD-3 antibodies, lipopolysaccharides, GM-CSF, IL-4 and IL-10 [25, 26].

The role of the PD-1 - PD-L1 axis in human carcinomas was first recognized in 2002 [26]. The mechanisms of T-cell inactivation is schematically illustrated in figure 2.
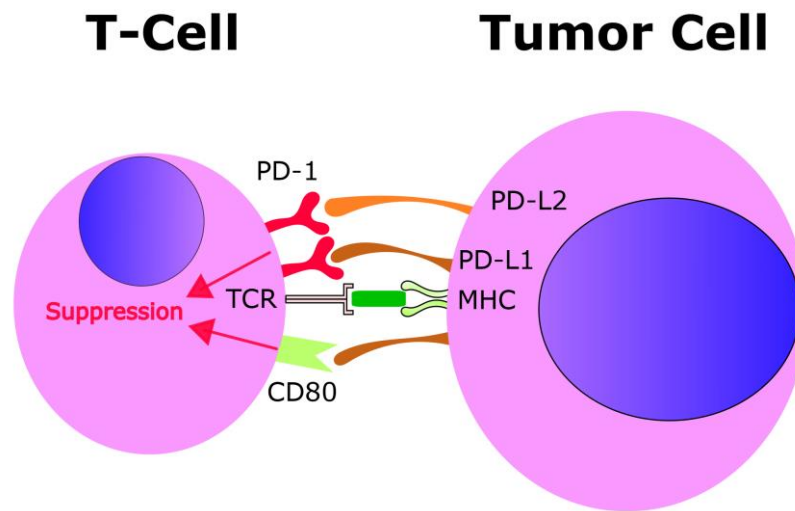
Figure 2:
Scheme of T-cell and tumor cell interaction via the PD1-PD-L1 axis: Beside binding of PD1 to PD-L1 or PD-L2 MHC interaction and coupling to CD80 on the T-cell surface is required for suppression of the T-cell mediated immune reaction.

However, while the expression of PD-L1 is easily quantifiable through mRNA techniques or immunohistochemistry, making a connection to patient characteristic and outcomes has not been as straight forward as one might expect.

PD-L1 expression and clinicopathologic characteristics of NSCLC

Validity of expression of PD-L1 in NSCLC specimens taken from Paraffin-embedded tissue by IHC is evidenced by studies using mRNA assays and IHC complementarily [27]. Although these results correlate to a high degree, others have found that post-transcriptional control of PD-L1 expression plays a role and could explain differences between mRNA and protein expression [28].

In the first study from 2004 to assess the relationship between PD-L1 expression in NSCLC and clinicopathological parameters, the authors reported insignificant results for any tested parameter including histologic subtype, survival, smoking and histological grading in a relatively small cohort of 52 patients [29]. Subsequently, it took until 2011 for the next similar study to be published.

Entity

Looking more closely at the results of these studies results have not been unequivocal. For example, the extent to which tumor cells in NSCLC show PD-L1 expression has been reported to be between 5,1% and 65,3% (Table 1). While some authors find higher expression of PD-L1 in squamous cell carcinoma (SCC) [27, 30], others report higher expression in adenocarcinoma

(LAC) [31, 32]. Here we tried to combine results of studies that supplied data on PD-L1 expression and histologic entity using a chi-squared table (Table 2). Since a culprit of many studies is small sample size we sought to combine comparable works irrespective of scoring method or antibody type, because the relative aspect of comparison between histologic entities is sustained. We found that across all reported studies the percentage of PD-L1 positive tumors was 28,5% for LAC and 24,1% for SCC. Taken together, PD-L1 expression is significantly lower in SCC compared to LAC using a chi-squared table (p = 0,008, Table 2). Still, response rates to inhibition of the PD-1/PD-L1 axis by antibodies have been observed in different Anti-PD-1 and Anti-PD-L1 trials that showed no significant differences between NSCLC histologic entities [33–35].

Table 1: Reported studies of PD-L1 in NSCLC, p-values for statistical test examining differential PD-L1 expression in LAC and SCC; Schmidt et al. did not discern between non-squamous and LAC

Author	N	Histology	TNM	%+	% LAC	% SCC	p
[29]	52	NSCLC	I-IV	27,2%	42,9%	54,8%	0,4
[36]	208	NSCLC	I-IV	65,3%	30,8%	27,3%	0,757
[32]	109	NSCLC	I-III	53,2%	65,2%	44,4%	0,032
[27]	340 (Greek)	NSCLC	I-IV	24,8%	22,6%	24,3%	0,88
[27]	204 (Yale)	NSCLC	I-IV	36,1%	27,5%	56,7%	0,004
[37]	163	LAC	I		39,9%		
[38]	214	SCC	I-IV			19,6%	
[39]	143	LAC	I-IV Asian		49,0%		
[31]	125	NSCLC	IV Italian	55,3%	63,4%	30,4%	0,005
[40]	331	SCC	I-III Asian			26,9%	
[41]	681	NSCLC	I-III Caucasian		5,1%	8,1%	0,14
[30]	274	NSCLC	I-III Caucasian	24,0%	20,0%	28,0%	0,089
[42]	332	NSCLC	I-IV	23,8%	20,0%	25,9%	0,24

Table 2: Chi-Squared analysis of reported studies of PD-L1 expression according to histological NSCLC entity

Observed	PD-L1+	PD-L1-	Total	PD-L1+%
LAC	398	999	1397	28,5%
SCC	351	1104	1455	24,1%
Total	749	2103	2852	
Expected				
LAC	367	1030		
SCC	382	1073		

Histological grading

In a meta-analysis encompassing six tissue microarray based PD-L1 expression studies, the authors showed that across this selection of publications there was a positive correlation between PD-L1 expression and histological grading in NSCLC irrespective of histologic entity [43].

Mutational load

While the amount of somatic mutations in cancer cells is thought to be unrelated to PD-L1 tumor expression, the role of somatic mutations in patients who have been treated with Nivolumab or Pembrolizumab therapy has been found to correlate with response to immunotherapy. According to two independent studies higher nonsynonymous mutation burden correlates with longer progression-free survival and a higher objective response rate in Anti-PD-1 treatment [44, 45]. Although PD-L1 expression and mutational burden is not correlated, high mutational load and PD-L1 tumor expression in combination are associated with the highest response rates. This has been linked to the effects of smoking on the mutational burden and generation of a broader range of neoantigens and therefore antigenicity and immune response.

Smoking

Interestingly, higher rates of response to Anti-PD-1 and Anti-PD-L1 therapy in smokers have been reported in clinical trials for Anti-PD-1 and Anti-PD-L1 [34, 46]. In retrospective analysis some groups show no correlation between PD-L1 expression and smoking, others show lower PD-L1 expression in smokers and again others even an inverse relationship [36, 47]. Since correlation between histological grade and smoking has been confirmed for NSCLC [48], the parallel relationship of histological grade and PD-L1 expression should be studied carefully with regards to smoking as well as mutational burden in order not to be confounded.

Driver Mutations

As shown by two authors, EGFR mutations seem to be associated with a higher expression of PD-L1 and response rates to tyrosine kinase inhibitors are higher in patients with PD-L1 expressing tumors [31, 47]. Also, treatment of EGFR-mutant NSCLC cell lines with a EGFR tyrosine kinase inhibitor (Erlotinib) can result in a marked down-regulation of PD-L1 as determined by flow-cytometry, suggesting a connection of the expressional pathway of PD-L1 and EGFR signaling [47].

Similar results have been published for an ALK mutated T-cell lymphoma cell line showing upregulation of STAT3 mediated expression of PD-L1 [49].

Still, responses to Anti-PD-1 and Anti-PD-L1 treatment seem to occur regardless of ALK or EGFR mutation status but it is of clinical importance to differentiate these tumors and clinical trials have excluded patients eligible for treatment with EGFR or ALK targeted therapies [34] It seems noteworthy that in at least one trial EGFR mutant NSCLC patients seemed to have a favorable outcome in the docetaxel group as opposed to the Anti-PD-1 treatment although the results were insignificant [50].

For squamous NSCLC the loss of PTEN and therefore an uncontrolled PI3K pathway also lead to an upregulation of PD-L1 expression [51]. Considering the mounting evidence of at least a partial relationship between the expression of PD-L1 and some signaling pathways further watchfulness in this space is warranted.

Prognosis

Several studies and meta-analysis have looked at the power of PD-L1 expression to effectively prognosticate patient survival, but unanimous consent about evidence of prognostic impact has not been established. While some studies argued that PD-L1 expression is associated with a negative prognosis [32], others have argued that it may even indicate a beneficial clinical course [52]. In fact, meta-analyses have consistently shown very poor prognostic potential [43, 53]. In the future studies regarding PD-L1 in combination with other markers such as PD-1 and the tumor microenvironment might help elucidate the field.

History of Anti-PD-1 treatment of NSCLC

Nivolumab was first to receive regulatory approval for second-line treatment of advanced squamous NSCLC irrespective of PD-L1 expression by tumor cells in March 2015 after already having been approved for the treatment of recurrent melanoma in 2014. Approval for advanced non-squamous NSCLC followed in October 2015. Pembrolizumab seemed like a conservative approach because it required an expression of the PD-L1 ligand of PD-1 of at least 50% of tumor cells when it received its FDA approval in October 2015. While the preliminary results of both studies were indicative of predictive potential of PD-L1, the amount of responses to therapy in PD-L1 negative tumors were equally impressive although seemingly counterintuitive. However, approval for Nivolumab was not granted for NSCLC as a first-line treatment after progression-

free survival was not superior to standard chemotherapy with 1% of tumor cells expressing PD-L1 by IHC as marker of eligibility for Anti-PD-1 therapy [54]. Merck pursued a different strategy with Pembrolizumab as a first-line monotherapy for advanced NSCLC, as it requested tumor expression of PD-L1 in at least 50% of cells. While both studies showed lower toxicity for respective immunotherapies, only for Pembrolizumab and selection for high tumor PD-L1 expression significant survival benefits was shown. This lead to the approval of Pembrolizumab as first-line therapy for NSCLC with expression of PD-L1 ≥50% as determined by the companion IHC scoring kit (PD-L1 IHC 22C3 pharmDx assay, DAKO) and no EGFR mutation or ALK fusion in October 2016 in the United States and in the EU in December 2016. Likewise, it will receive first-line status in locally advanced or metastatic disease with expression of PD-L1 $\geq 1\%$ and no evidence of targetable molecular aberration or prior treatment of a driver mutation. Nevertheless, even if PD-L1 expression is very high, the response rate in advanced NSCLC was "only" 45,2%. [55].

Anti-PD-L1 therapy

Atezolizumab became the third inhibitor of the PD-L1/PD-1 pathway to be approved for standard treatment in second line advanced NSCLC. It was the first specific targeting PD-L1. The concept of therapeutic interruption of the PD1-PD-L1 axis is shown in figure 3.
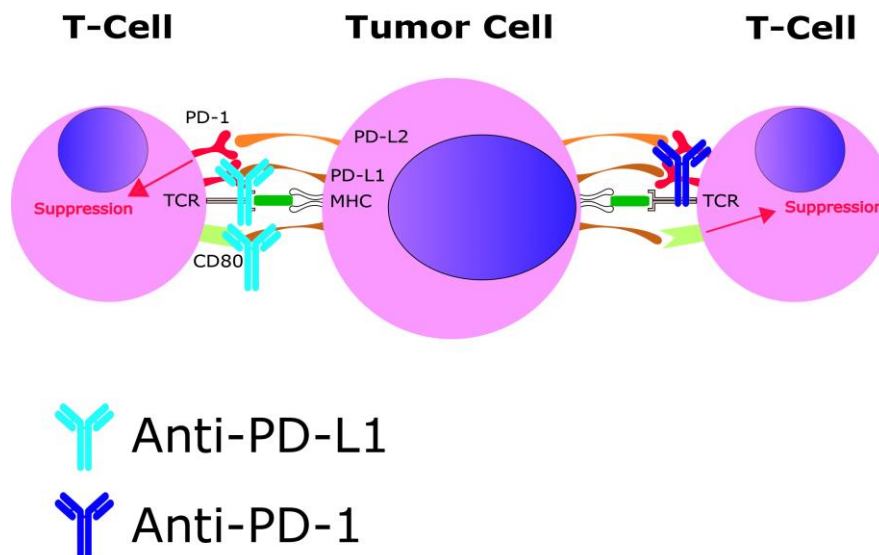

Figure 3:
Concept of therapeutic interruption of the PD1-PD-L1 axis to disrupt the tumor cell induced downregulation of the T-cell mediated cytotoxic immune response. While anti-PD-L1 antibodies (e. g. atezolizomab, durvalumab) block PD-L1 on the surface of tumor cells, anti-PD1 antibodies (e. g. nivolumab, pembrolizumab) interfere with T-cells directly.

Ventana's SP142 is the antibody being used in conjunction with Atezolizumab, however PD-L1 testing is not required for prescription.

As shown in Anti-PD-1 treatment, PD-L1 expression correlated but was not conditional for therapeutic response [56]. Like for the Anti-PD-1 treatments, patients with no or very low expression of PD-L1 showed paradoxical response to Anti-PD-L1 treatment with Atezolizumab and greater efficacy in those with higher expression.

Notably, in recently released study results of the Atezolizumab trial in 1202 patients with non-squamous NSCLC, response to therapy was unrelated to tumor cell PD-L1 expression, but improved in patients with a T-effector cell gene expression signature, providing evidence that an already ongoing immune response against tumor cells is enhanced by Anti-PD-L1 therapy [57].

Another Anti-PD-L1 antibody which was approved by the FDA for locally advanced, unresectable NSCLC for patients who have not progressed following radiochemotherapy in February 2018 is Durvalumab. Progression-free survival to Anti-PD-L1 therapy was irrespective of PD-L1 expression before radiochemotherapy, but expression levels were not reassessed [58]. The effects of radiotherapy on the tumor mutation burden and possibly increased PD-L1 expression should be considered when evaluating response rates to immunotherapy since paradoxically there are increased response rates to combination of radiation and immunotherapy and reports of decreased PD-L1 expression in post-radiation specimens of NSCLC [59].

Combination immunotherapy

A search query for "PD-L1" and "combination" on www.clinicaltrials.gov restricted to recruiting and active trials bring up 273 distinct clinical phase I, II and III studies investigating the combinatorial potential of immunotherapies targeting PD-1 and PD-L1 across many different solid and hematologic neoplastic entities and 49 distinct trials in non-small cell lung cancer designated trials.

For example, Durvalumab is currently being tested in combination with tremelimumab, an Anti-CTLA-4 antibody and also a member of the class of "checkpoint inhibitors", for NSCLC [60]. There are also many studies ongoing that combine Anti-PD-L1 and Anti-PD-1 therapies with other novel immunotherapeutic agents like Anti-Lag3 and Anti-TIM-3 antibodies, further disinhibiting the immune system to attack cancer cells. Other approaches like cancer vaccines, oncolytic viruses and CAR-T therapies look to leverage the immune response by combining checkpoint inhibitors and antigenicity driven mechanisms. It would be very interesting to see studies examining complete inhibition of the T-cell suppressive effects of PD-L1 and PD-1 as illustrated in figure 3. Importantly, Anti-PD-L1 treatment does not only interfere with binding of PD-L1/PD-L2 with the PD-1 receptor on T-cells but also the binding of PD-L1 and CD80 (B7.1), which is also responsible for T-cell suppression [61]. Therefore, investigation of a combinatorial Anti-PD-L1

and Anti-PD-1 antibody may be warranted. Taken together all combinatorial trials will possibly help further our understanding of underlying mechanisms of immune escape mechanisms.

Side effects

Side effects have been shown to be almost equivalent between Anti-PD-1 and Anti-PD-L1 treatments but less toxic than Anti-CTLA-4 and Chemotherapy. A growing concern in the field refers to reports of hyper progressive diseases among patients receiving immune checkpoint inhibitors. A French study reports of 9% of patients suffering from an accelerated progression of disease after initiation of therapy with Anti-PD-1 or Anti-PD-L1 therapy [63]. Importantly this data is differentiated from reports of delayed onset of response or pseudo progression by tumor inflammation reported in earlier studies [64]. Mechanistically hyper progressive disease cannot be easily explained as it occurred across entities, and burden of tumor, but was associated with high age and worse outcome. Also, as could be suspected from previously mentioned mouse models, reports of immunotherapy induced distinct autoimmune diseases start to emerge as use of these new treatments start to become more prevalent [65].

Considerations

One of the key differences to other established oncological predictive biomarkers like HER2 and ER in breast cancer and EGFR or ALK aberration in NSCLC is that the expression of PD-L1 in cancer tissue is probably non-homogenous, inducible and therefore subject to change making false-positive as well as false-negative estimations of expression possible [66]. Consequently, it is most important to realize that response to PD-L1 therapy can merely be loosely predicted but not guaranteed by the analysis of PD-L1 expression so far. Treatment failure despite abundant PD-L1 expression, acquired resistance and paradoxical responses in PD-L1 negative patients should be expected. Thus, it will be challenging to make helpful statements in a clinical setting.

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