

Programmed death 1 (PD-1) and its ligand (PD-L1) as a new frontier in cancer Immunotherapy and challenges for the Pathologist: state of the art

M. CALLEA, F. PEDICA, C. DOGLIONI
Pathology Unit, San Raffaele Scientific Institute, Milan, Italy

Key words

PD1/PD-L1 • Immunotherapy • Cancer

Summary

The interest in better understanding the immune-microenvironment and tumor cells crosstalk, recently leads to focus on immune checkpoints role, notably on PD-1/PD-L1 axis.

The current backdrop concerning cancer immunotherapy is constantly evolving and new biomarkers still need to be granted in this dynamic context.

This review tries to get lights on PD-L1 complex scenario mainly focusing on troubling issues in assessing this marker in daily practice.

It's still necessary to look deeper into this matter in order to make easier the pathologists-oncologist interaction.

Introducing PD1/PDL1 pathway

In the last few years, the fighting against cancer has focused on immunotherapy, driving efforts on several immune checkpoint inhibitors in order to reinvigorate and enhance the immune response against tumor cells. Cancer is able to find several strategies to escape from the endogenous antitumor immune response "silencing" T-cells functions. For this reason the interest in understanding how the immune microenvironment and tumor cells interact with each other, has incredibly increased¹. Over the past few years, a large number of studies focused on Programmed cell death 1 (PD-1) and its ligand Programmed cell death ligand 1 (PD-L1/B7-H1/CD274) because of the involvement of this pathway in down-regulating intensity and duration of T-cells immune responses². PD-1 was first identified in 1992³ and later the PD1/PD-L1 pathway has been recognized as an immune check-point; once activated, this pathway inhibits T-cells proliferation and survival and scrapes the effector functions such as cytotoxicity and cytokine release. It is also involved in promoting the differentiation of CD4+ T-cells into FOXP3+ regulatory T-cells further inhibiting effector T-cells functions⁴.

PD-1 is a member of the immunoglobulin gene family and several studies demonstrated how it is expressed on the surface of activated T cells, activated B cells, regulatory T-cells (Treg) and natural killer (NK).

It has two ligands, PD-L1 and PD-L2 and when the T-cell receptor PD-1 binds to its ligands on antigen presenting cells (APC), the inhibitory pathway is activated leading to T-cells suppression (Fig. 1).

PD-L1 (B7-H1 or CD274) is a cell surface glycoprotein and it has been demonstrated how it is basically express in sites like placenta tonsil and retina, all implicated in immuno tolerance mechanism; the protein can also be expressed on hematopoietic cells (dendritic, myeloid, T and B cells), non-hematopoietic cells and on tumor cells⁵.

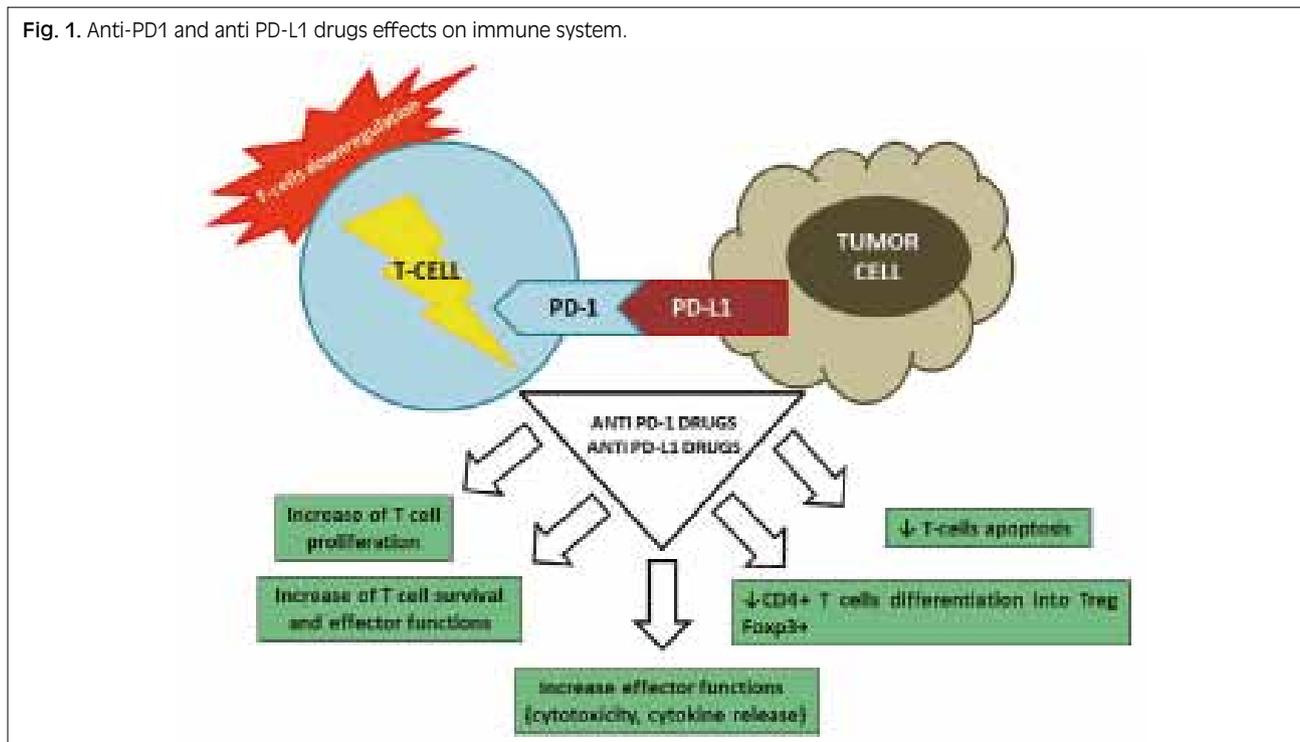
B7-H1 mRNA is expressed in almost all human tissues but cell membrane protein expression is confined to specific groups of cells. Then, it is conceivable that PD-L1 mRNA regulation is normally depending on post-transcriptional regulation. On the other side, the protein can be expressed on different types of cancer cells⁶.

Human PD-L1 gene (CD274) is located on chromosome 9p24 and it's made by seven exons: the first one is a non-

Correspondence

M. Callea, Pathology Unit, San Raffaele Scientific Institute, via Olgettina 60, 20132 Milan, Italy - E-mail: callea.marcella@hsr.it

Fig. 1. Anti-PD1 and anti PD-L1 drugs effects on immune system.



coding exon, back to back there are the signal sequence (exon 2), the IgV-like and the IgC-like domains (exon 3 and 4 respectively). Exons 5 and 6 incorporate the transmembrane and the intracellular domains.

CD274 gene encodes for a type I-transmembrane glycoprotein of 290 amino acids and it is composed by an extracellular domain and a short intracellular tail, this latter made by 31 amino acids. The majority of the transmembrane protein is extracellular, including the PD-1 binding domain⁷.

PD-L2 (B7-DC or CD273) expression is induced more strongly by interleukin 4 (IL-4) than INF gamma, and it is mainly expressed on activated dendritic cells and some macrophages⁸.

PD-1 is overexpressed on CD4+ and CD8+ tumor infiltrating lymphocytes (TILs) in many tumor types: PD-L1 expression in tumor cells induces the activation of PD1/PDL1 pathway, facilitating immune evasion and correlating with tumorigenesis and invasiveness: suppressing the PD1/PD-L1 pathway it is therefore possible to restore the function of exhausted CD8+ T-cells⁹.

Recent clinical trials have in fact demonstrated that it's possible to induce durable remission in several tumors blocking the PD1/PD-L1 axis with anti-PD-1 or anti-PD-L1 antibodies and that an objective clinical response was closely associated with PD-L1 expression in tumor cells¹⁰. Therefore, inhibition of the PD1/PD-L1 axis is becoming an exciting approach to consolidate host immunity in many different type of cancer^{11,12}.

PD-L1 expression on tumor cells and on hematopoietic cells, which are part of tumor microenvironment, can be regulated by innate and adaptive immune resistance. The

innate mechanism consists in PD-L1 expression induced by oncogenic pathway alteration as it has been demonstrated for example in glioblastoma which is characterized by loss of PTEN¹³, in lymphomas and lung carcinoma both characterized by constitutive activation of ALK signaling¹⁴; PD-L1 expression could also be induced by genetic abnormality like, for example amplification¹⁵. In the adaptive immune resistance cancer is able, under INF gamma stimulation, to co-opt the natural physiology of the PD-1 pathway, thus silencing the immune system^{6,9} (Fig. 2).

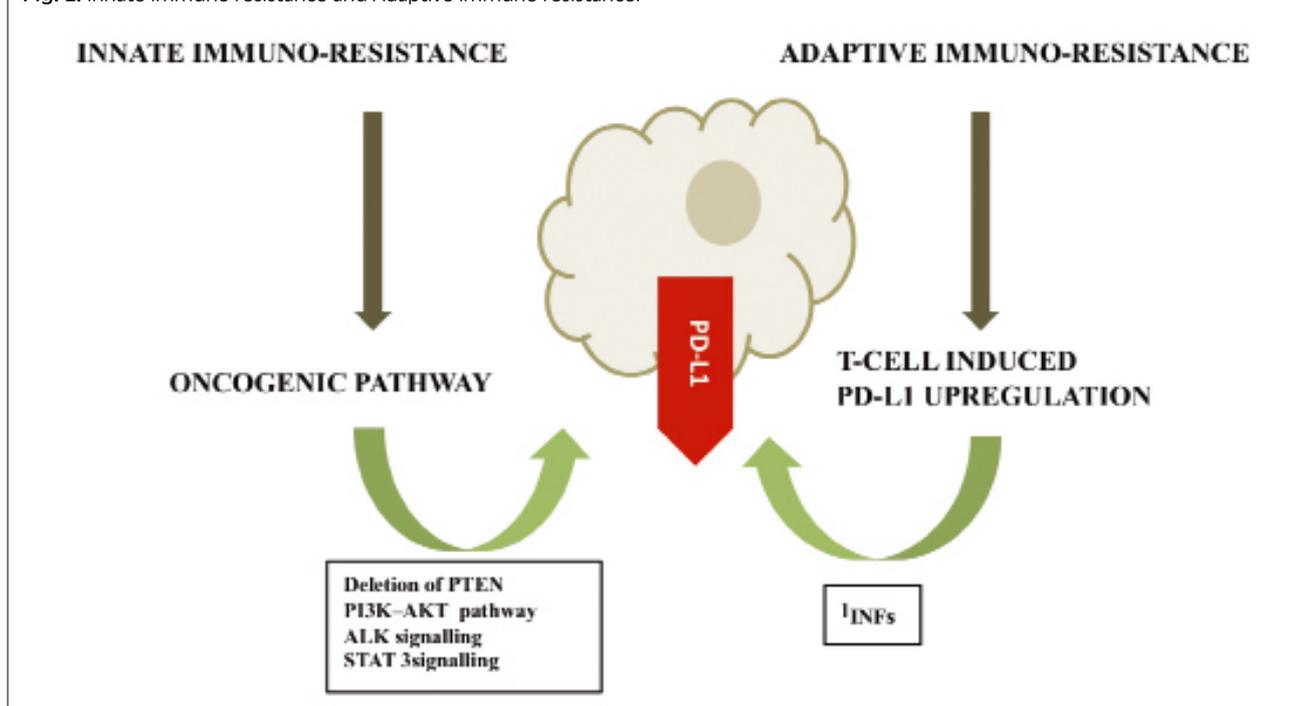
A literature meta-analysis have highlighted how PD-L1 expression in tumor cells of different neoplasms correlates with a significant clinical response when treated with anti PD-1/PD-L1 agents¹⁶.

Troubling issues

HOW TO EVALUATE PD1-PD-L1 AXIS

Although mechanisms through which tumor cells express PD-L1 are quite well known, to date several studies demonstrated how only a small subset of patients take advantage of a PD1/PD-L1 immunotherapy regardless of the PD-L1 expression on tumor cells^{17,18}. The problem is at least in part due to a lack of uniform methods for PD-L1 detection and evaluation. Therefore, criteria for selecting patients who are candidate to immunotherapy and benefit from it, are still debated. Currently, the goal is to identify the target patients population which can really get benefits from this immunotherapy considering its great clinical significance but also its toxicity¹⁰.

Fig. 2. Innate immune resistance and Adaptive immune resistance.



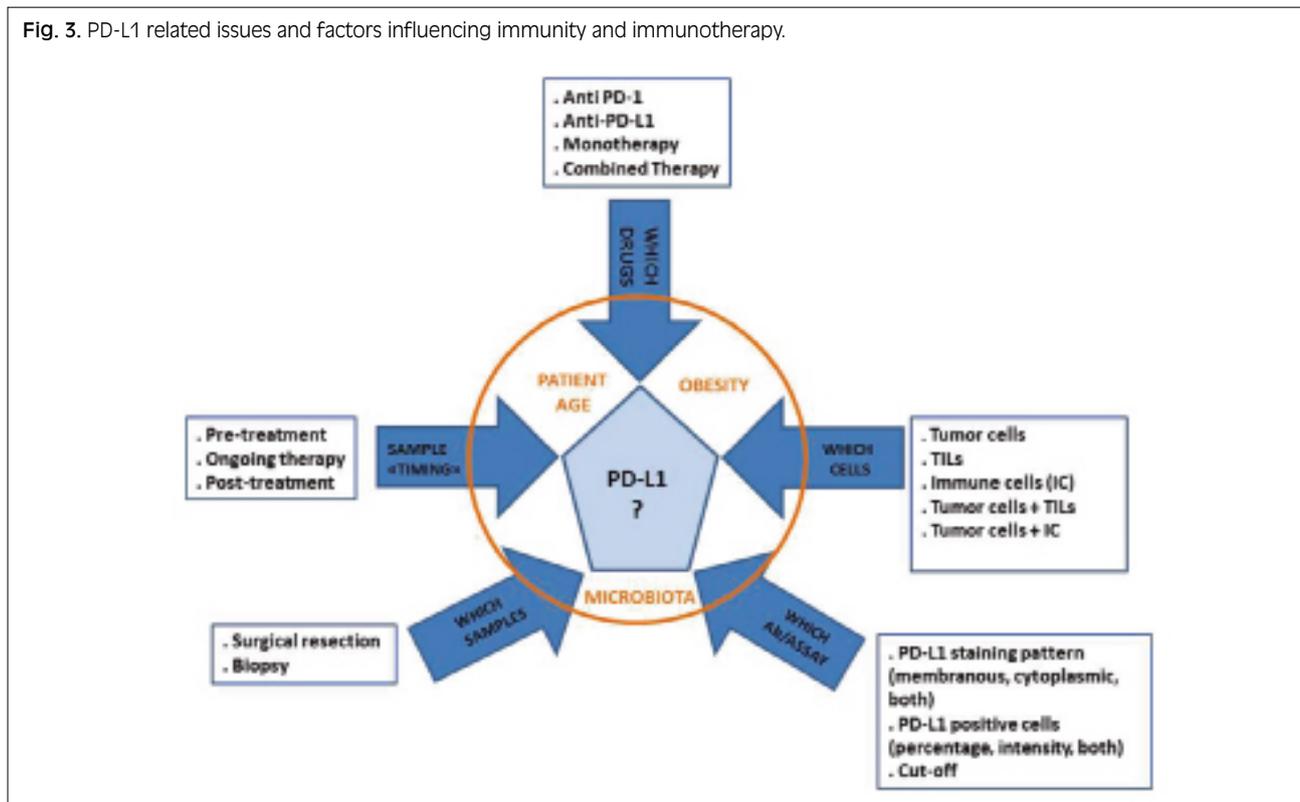
Specifically, issues related to PD-L1 investigation concern not only materials and methods used to detect PD-L1 expression but also results evaluation; these two aspects are strictly related. The concept that just a subgroup of patients respond to this innovative type of immunotherapy is not only due to a lack of PD-L1 detecting methods standardization but also to patients “variables” as age, weight and diet. Recent studies focused on these important patients aspects emphasizing how age, weight and microbiota can deeply influence immune reaction to cancer and therefore, the response to immunotherapy¹⁹. Issues concerning PD-L1 are summarized in Figure 3.

Immunohistochemistry represents the most widely accepted and used method for PD-L1 assessment, but we have to face with the use of different antibodies and different staining protocols that consequently affect the way to assess PD-L1 expression and results evaluation. In literature, different antibodies against the same protein but specific for different protein epitopes, are reported and tested, generating low homogeneity, low reproducibility and discordant results²⁰. Indeed, the targeted epitope recognized by the antibody used, affects the PD-L1 staining and scoring evaluation. In recent studies, different antibodies have been tested on formalin fixed paraffin embedded (FFPE) tissues targeting either the extracellular protein domain or the intracytoplasmic tail. Several anti-PD-L1 antibodies used in different studies, get to a mix cytoplasmic and membranous staining^{21,22} making the PD-L1 score evaluation tricky. Mahoney et al.¹² compared the immunohistochemical staining of five anti-PD-L1 antibodies, two of these (7G11 and 9A11) produced by

their own laboratory and three commercially available (015, E1L3N and SP142). Two antibodies (7G11 and 015) target the extracellular protein domain, the others the cytoplasmic tail. They stained five different tumor types and demonstrated different staining according to the antibody-target type (extracellular or cytoplasmic) emphasizing how antibodies against the cytoplasmic tail of the PD-L1 protein can better outline the membranous pattern²³. Since it is well demonstrated in multiple studies how PD-L1 membranous expression on tumor cells or infiltrating immune cells is correlated to a better chance of response to anti PD1 drugs, distinguishing between membranous and cytoplasmic staining is a difficult tightrope and it still represents an enormous limit^{24,25}. Furthermore there is still no a universally recognized cut-off establishing a positive test result and there is no uniformity even in term of scoring methods (percentage of positive cells, staining intensity, H-score). Moreover, many studies focused on different target cells for PD-L1 assessment such as tumor cells, infiltrating tumor immune cells or both. In fact, it’s well known that not only tumor cells but also infiltrating mononuclear immune cells can express PD-L1. In particular, the number of infiltrating T-cells and the proportion of T-cells positive for PD-L1 or PD-1 are considered indices of therapeutic response to PD-L1/PD-1 inhibitors in several tumors²⁶⁻³⁰.

Comparing these studies, we could say that it’s still not clear which is the best approach for evaluating the immune context and further investigation should be considered to better define its role towards a personalized treatment.

Fig. 3. PD-L1 related issues and factors influencing immunity and immunotherapy.



PD-L1 IS A “HETEROGENEOUS” AND “DYNAMIC” MARKER

Concerning heterogeneity, PD-L1 is expressed on different cells type and the staining can be detected in the cytoplasm or on cell membrane or both; furthermore in some cases, it has been demonstrated how PD-L1 expression within the same tumor can be variable according to tumor differentiation grade^{10,31}. Moreover, in tumor cells PD-L1 expression can change during and after treatment influenced by the administrated drugs and by immune state. Indeed, chemotherapy or target therapy may induce PD-L1 expression in immune therapy-naive tumors³².

Studies conducted on cell lines from Non-Small Cell Lung Carcinoma (NSCLC) showed the effects of different chemotherapies on PD-L1 expression; it has been highlighted how Doxorubicin can down-regulate membranous PD-L1 expression on cancer cells and how, on the other side, Etoposide and Paclitaxel are able to induce PD-L1 expression³³. Even the PD-L1 expression on tumor infiltrating immune cells can be modified by treatment. In summary, all of these findings suggest the necessity to better investigate which kind of sample should be collected (biopsy versus resected sample), the collection “timing” (should we follow PD-L1 expression changes over time to set and adjust therapy time after time?) and the best method to evaluate it (immunostaining, western blotting or RT-polymerase chain reaction- RT-PCR-).

Another sticking point still not yet well investigated concerns the relation between PD-L1 expression in primary tumors and their corresponding metastases. A study

from Jilaveanu et al.³⁴ conducted on a series of primary Clear Cell Renal Cell Carcinoma and matched metastases showed greater PD-L1 expression in metastatic tumor than primaries detecting PD-L1 expression using an Automated Quantitative Analysis (AQUA) method on a tissue micro-array (TMA). Discordance in PD-L1 expression between primary and metastatic sites has also been demonstrated in another recent study in 20.8% of a series of primary Clear cell RCC and corresponding metastases³⁵. Such studies highlight how might be significant to assess PD-L1 expression not only on the primary tumor but also on metastatic lesion.

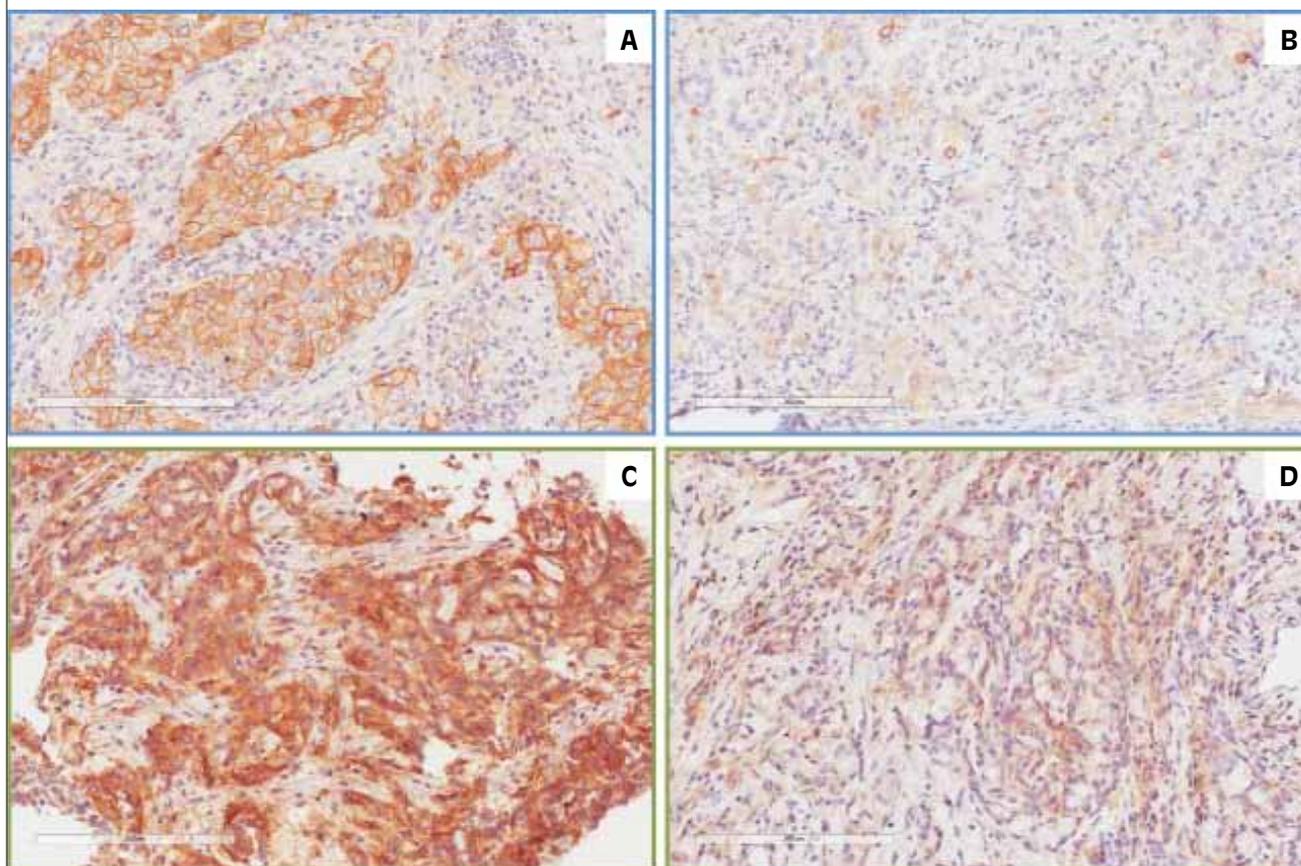
In conclusion, lack of standardized methods and PD-L1 intrinsic complexity led to an intricate “PD-L1 landscape” which is still quite difficult to interpret^{36,37}. Other studies and data are needed to clarify the role of PD-L1 as predictive biomarker using immunohistochemistry technique.

In figures 4, 5, 6, 7 are illustrated some representative imagines of FFPE samples immunostained with anti PD-L1 antibody (clone SP142, Spring) set up in our institution (S. Raffaele Hospital, Milan). These pictures show examples of primary pulmonary and renal primary tumors with matched metastases immunostained with the SP142 clone.

Anti-PD1/PDL1 agents, ongoing studies and PD-L1 companion diagnostics

By blocking the PD1/PD-L1 pathway through anti PD-1 or anti PD-L1 antibodies it is possible to get a durable

Fig. 4. FFPE samples immunostained with anti-PD-L1 antibody (clone SP142, Spring). A,B. Images of primary lung adenocarcinoma with positive tumor component (A) and negative PD-L1 tumor component but positive PD-L1 inflammatory immune cells (B). Matched lymph node metastases with positive tumor component (C) and negative tumor component (D).



remission in different type of tumors³⁸; different specific inhibitors are already in clinical practice and many more are under investigation and “protagonists” of the ongoing clinical trials.

Currently drugs already in place in clinical practice, approved in 2014 by the US Food and Drug Administration (FDA) are two PD-1 inhibitors, Nivolumab (Opdivo, Bristol-Myers Squibb) and Pembrolizumab (Keytruda, Merck) for unresectable or metastatic melanoma and for squamous and non squamous non-small cell lung cancer (NSCLC) treatment³⁹.

FDA extended indications to the use of Nivolumab in second line treatment for patients with metastatic Renal cell Carcinoma on the basis of the CheckMate -025 phase III clinical trial demonstrating a prolonged survival with nivolumab, as compared with everolimus, in a cohort of 821 patients with advanced Renal cell Carcinoma⁴⁰.

In addition, in October 2015 FDA proposed two different immunohistochemical (IHC) assays for detecting PD-L1 expression evaluated on formalin-fixed, paraffin-embedded NSCLC samples. The established assays are PD-L1 IHC 22C3 and PD-L1 IHC 28-8 (Dako) for Pembrolizumab and Nivolumab, respectively⁴¹.

Concerning Pembrolizumab, the approved companion diagnostic assay envisions a cut-off regarding positive membranous staining in $\geq 50\%$ tumor cells. This cut-off

has been established on the basis of the KEYNOTE-001 results study showing a higher response rate and longer progression-free and overall survival in NSCLC patients with $\geq 50\%$ positive tumor cells⁴¹.

For Nivolumab the framework is different. Several trials demonstrated a significant response rate in PD-L1 positive NSCLC; on the other hand there is a relevant percentage of patients that showed response to Nivolumab with PD-L1 negative tumor. In summary, a patient with a PD-L1 negative tumor can anyway benefit from therapy^{42 43}.

Despite of several ongoing trials testing Nivolumab, so far no approved assay as a companion diagnostic has been developed but FDA speaks only about a sort of “complementary test” according to which a tumor should be considered “positive” when $\geq 1\%$ of neoplastic cells are positive for anti-PD-L1 28-8 (pharmDx)⁴⁴. Within the “immune checkpoint therapy” landscape, anti-PD-L1 drugs have to be included too. Actually Atezolizumab (MPDL3280) and Durvalumab (MEDI4736) are in Phase III study and the FDA has started to evaluate the possibility to administer Atezolizumab in patients with NSCLC expressing PD-L1 and whose disease gets worse during or after a prior standard therapy and in patients with metastatic bladder cancer expressing PD-L1. To date, Atezolizumab is being evaluated in

Fig. 5. FFPE samples immunostained with anti PD-L1 antibody (clone SP142, Spring). Images of primary Clear Cell Renal Cell Carcinoma (ccRCC) with an higher grade tumor component positive for PD-L1 (A) and a lower grade tumor component negative for PD-L1 (B). Matched adrenal gland metastases with PD-L1 positive tumor cells (C).

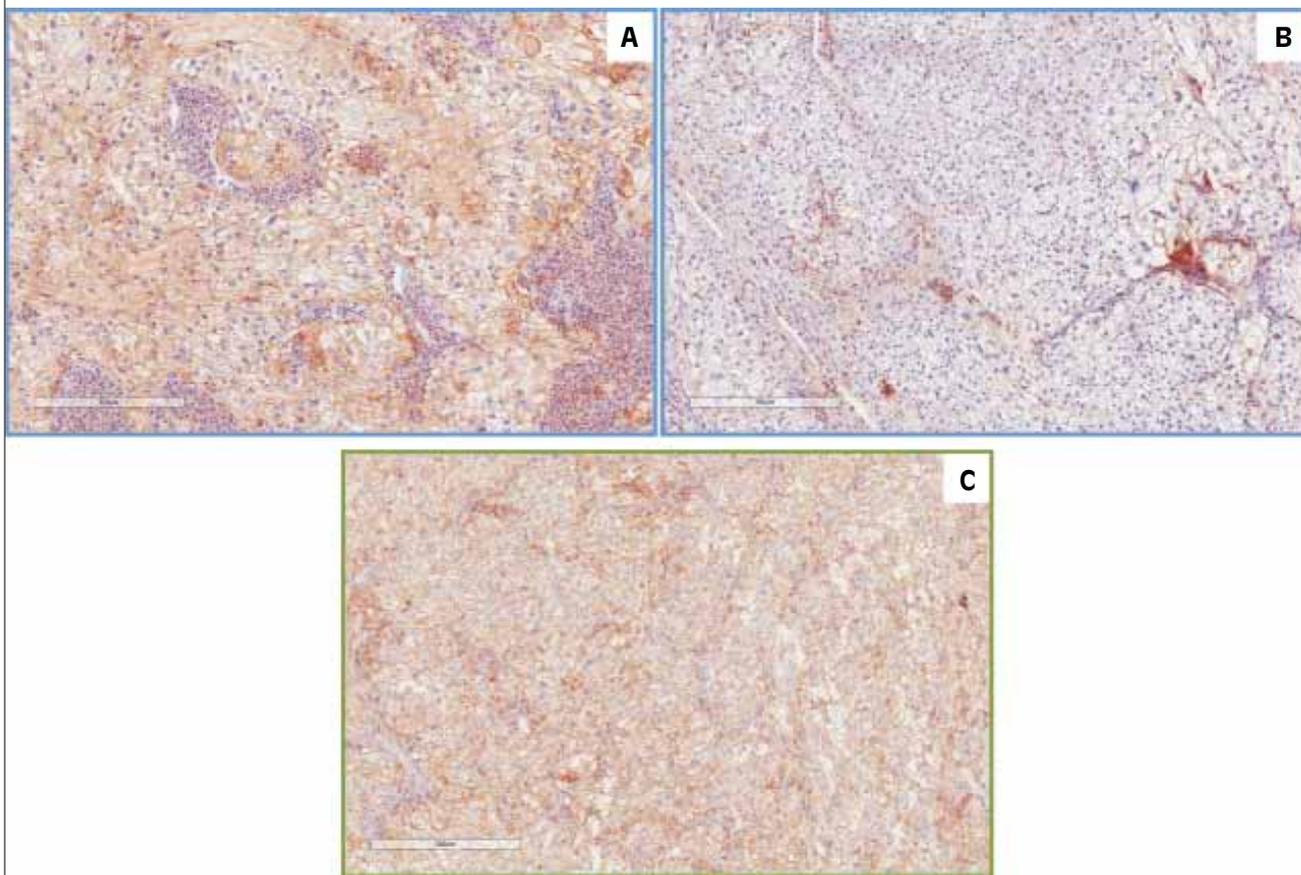
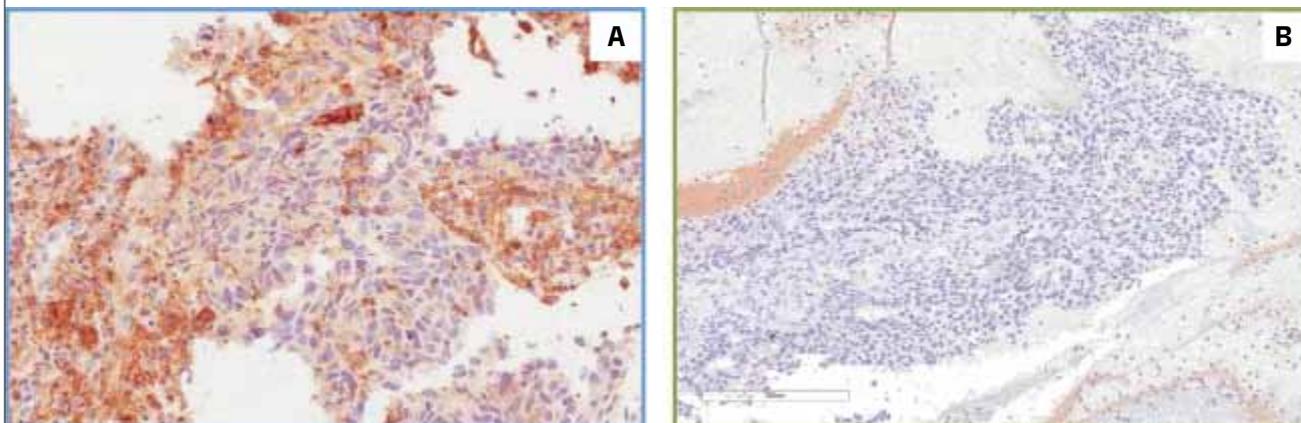


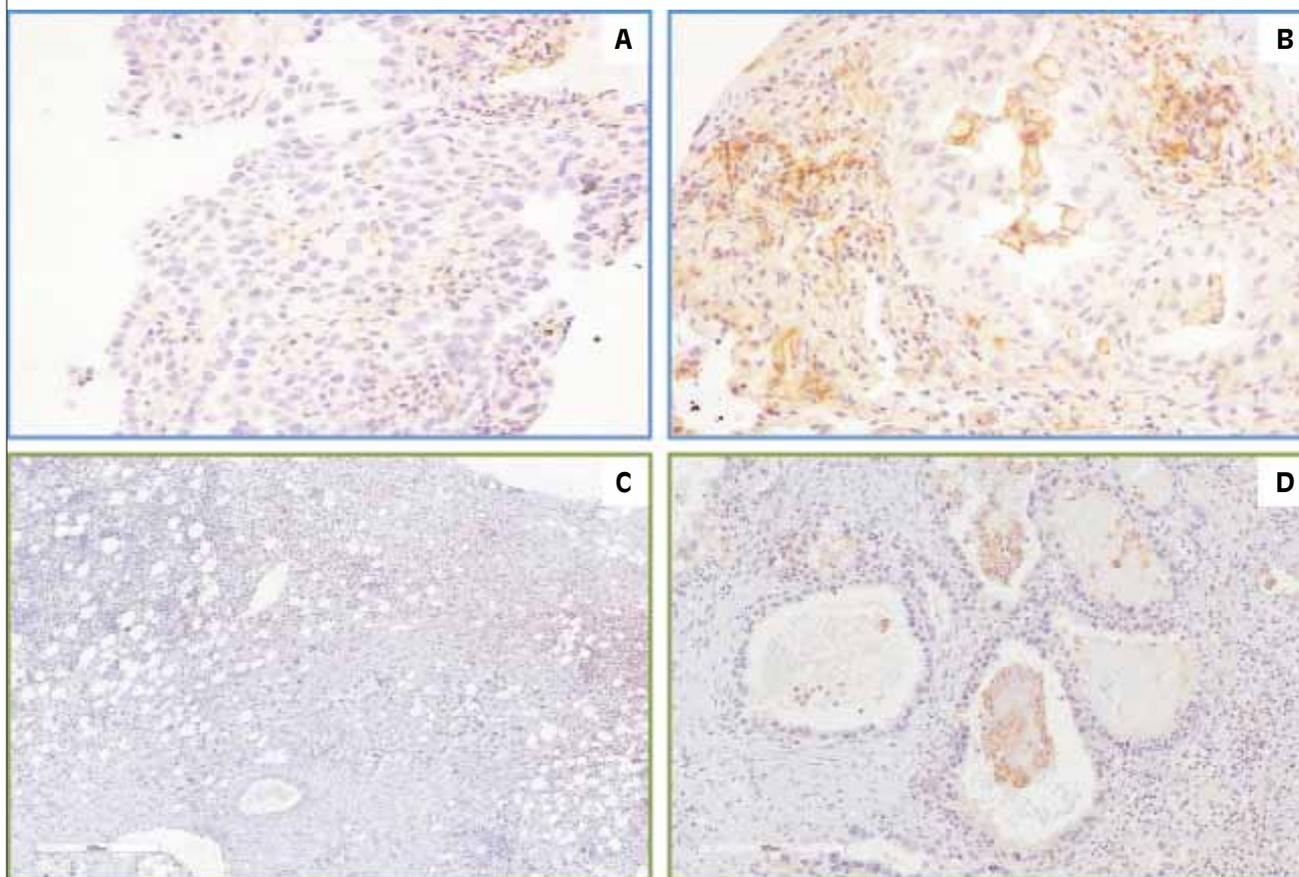
Fig. 6. FFPE samples immunostained with anti PD-L1 antibody (Clone SP142, Spring). A. Lung adenocarcinoma with negative PD-L1 tumor cells and positive PD-L1 inflammatory immune cells. B. Matched brain metastases with negative PD-L1 tumor cells.



at least 10 Phase III studies including NSCLC, bladder cancer, renal cancer and breast cancer⁴⁵. Relatively to Atezolizumab, which safety and effectiveness are currently being examined as mentioned above, the PD-L1 immunohistochemistry platform suggested is the Ventana SP-142 clone whose assay is still in development⁴⁶.

Atezolizumab is a humanized anti-PD- L1 monoclonal immunoglobulin G1 antibody which clinical activity has been demonstrated in several solid tumors⁴⁵. In these tumors Atezolizumab activity has been demonstrated to be related to PD-L1 expression on immune cells, and/or tumor cells predicting response to anti PD-L1 and anti PD-1 agents.

Fig. 7. FFPE samples immunostained with anti PD-L1 antibody (Clone SP142, Spring). A,B. Images of primary lung adenocarcinoma with negative PD-L1 tumor cells (A) and positive PD-L1 inflammatory immune cells (B). Matched bone metastase with negative PD-L1 tumor cells (C,D).



Concerning NSCLC in the POPLAR phase II trial Fehrenbacher and colleagues evaluated PD-L1 expression both on tumor cells and tumor infiltrating lymphocytes in patients with NSCLC after platinum based therapy, in order to assess efficacy and safety of Atezolizumab versus Docetaxel. They demonstrated that Atezolizumab significantly improved survival compared with Docetaxel and the improvement is correlated to PD-L1 expression both on tumor and immune cells⁴⁷.

For bladder cancer a significant phase II, single-arm, multicenter trial has been set up to evaluate Atezolizumab activity in patients with advanced urothelial disease pre-treated with platinum based chemotherapy. This study demonstrated how Atezolizumab is particularly effective in patients with elevated expression of PD-L1 on tumor infiltrating lymphocytes (TILs); PD-L1 expression on tumor cells was low and was not associated with objective response⁴⁸.

McDermott and Colleagues have recently showed that an increased expression of PD-L1 in tumor infiltrating immune cells of renal cell neoplasms but not of the neoplastic cells themselves, was associated with higher objective response rate (ORR)⁴⁹.

In those studies Investigators stained the formalin fixed-paraffin embedded samples with an anti-human PD-L1

rabbit monoclonal antibody (Clone SP142 from Spring Bioscience) targeting the intracellular protein domain of PD-L1; the staining was evaluated according to the Ventana SP142 PD-L1 immunohistochemistry assay. Giving the efficacy of Atezolizumab, it could be placed as a novel therapeutic solution blocking the PD-1/PD-L1 pathway⁴⁵.

Again, to better shed light on the potential predictive role of PD-L1 expression on tumor cells of different cancer types, Carbognin and colleagues⁵⁰ conducted an exciting metanalysis. They highlighted the differential activity of Nivolumab, Pembrolizumab and atezolizumab according to PD-L1 expression on tumor cells. They focused on twenty trials in different phases concerning Nivolumab, Pembrolizumab and Atezolizumab as treatment for patients with advanced melanoma, NSCLC and genitourinary cancer and whose tumor were tested for PD-L1 expression; as result, this study brings out the higher overall response rate (ORR) in patients with PD-L1 positive tumor cells treated with Nivolumab and Pembrolizumab. This study underlined how significant can be the drug's activity according to PD-L1 tumor cells expression.

Durvalumab is a high-affinity human IgG1 monoclonal antibody, selective in blocking the bond between PD-

Tab. I. Current state of PD-1 and PD-L1 inhibitors.

	Drug and Company	Antibody clone	IHC Assay	Antibody type	Target domain	IHC positive Cut-off	FDA diagnostic definition	Clinical Testing Phase
Anti-PD1	Pembrolizumab (Keytruda, MK-3475)	22C3	Dako	Mouse monoclonal	Extracellular	≥ 50% tumor cells	Approved as «Companion diagnostic» (October 2015)	FDA approved for advanced melanoma and metastatic NSCLC, see Ref*
Anti-PD1	Nivolumab (Opvivo, BMS-936558)	28-8	Dako	Rabbit monoclonal	Extracellular	≥ 1% tumor cells	Approved as «Complementary diagnostic» (October 2015)	FDA approved for advanced melanoma metastatic NSCLC and as a second line treatment for renal cell carcinoma after a failed anti-angiogenetic therapy , see Ref **
Anti-PD-L1	Atezolizumab (MPDL 3280 A) Genentech/Roche	SP142	Ventana	Rabbit monoclonal	Cytoplasmic tail	IHC 0: <1% IHC 1: ≥ 1% to ≤5% IHC 2: ≥5% to ≤10% Tumor cells and tumor infiltrating immune cells (Bladder, NSCLC, Breast)	In development	FDA «breakthrough designation» for advanced bladder cancer and NSCLC See Ref*** Ongoing clinical trials for melanoma, breast cancer, NSCLC,bladder cancer and renal cell carcinoma See Ref†
Anti-PD-L1	Durvalumab (MEDI-4736) AstraZeneca	SP263	Ventana	Rabbit monoclonal	Extracellular	≥ 25% tumor cells (NSCLC, head and neck squamous cell carcinoma)	In development	Durvalumab is being investigated in an extensive clinical trial programme, as monotherapy or in combination with tremelimumab, in NSCLC, head and neck, gastric, liver, pancreatic and bladder cancers See Ref††
Anti-PD-L1	Avelumab (MSB0010718C) In development by Merck-Pfizer	Expected	Expected	Expected	Expected	Expected	Expected	Phase I clinical trials for: Bladder, gastric, head and neck cancers, NSCLC, mesothelioma, ovarian cancer and renal cancer. Phase II clinical trial for: Merkel cell carcinoma Phase III clinical trial for: NSCLC See Ref†††

*<http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf>. Published August 6, 2014. Accessed December 3, 2015.
 **http://www.accessdata.fda.gov/drugsatfda_docs/label/2015/125554s012lbl.pdf
 ***Reichert JM. Antibodies to watch in 2016. mAbs 2016;8:197-204.
 †<https://clinicaltrials.gov>. search for: MPDL 3280A
 †† Breakthrough- Therapy-designation-by-US-FDA-for-treatment-of-patients-with-PD-L1-positive-urothelial-bladder-cancer-17022016.html
 ††† <https://clinicaltrials.gov>. search for: MSB0010718C

L1 with PD-1 and CD80. PDL-1 monoclonal antibody SP263 (Ventana) binds the extracellular domain of PDL-1⁵¹.

Only very recently it has been proposed a PD-L1 immunohistochemical diagnostic test developed by Ventana Medical Systems for use with Durvalumab, presented at the Translating Science into Survival conference in September 16-19, 2015 in New York⁵². In fact, the Authors optimized an anti-human PD-L1 rabbit monoclonal antibody (SP263) for use with Ventana OptiView DAB IHC Detection Kit on the automated BenchMark ULTRA platform, applicable and validated in formalin-fixed, paraffin-embedded samples of NSCLC. The staining was considered positive when the membranous staining was present in $\geq 25\%$ of tumor cells, whatever the intensity and with an inter-reader precision agreement of 97%. The Authors found out that PD-L1+ patients with these scoring system had a higher response rate compared with PD-L1- patients⁵².

Actually there are at least 4 on-going studies involving Durvalumab in advanced NSCLC and in 2 of them it was applied in association with other drugs⁵³.

At ASCO 2015, it has been presented an ongoing Phase 1/2, on multiple solid tumors including NSCLC, developed with multiple centers, in which Durvalumab has demonstrated prolonged response and acceptable tolerability⁵⁴.

More recently, another study in Phase 1b, was developed in 102 immunotherapy-naive patients and achieved objective responses in 23% of patients undergoing Durvalumab plus Tremelimumab (selective human IgG2 monoclonal antibody inhibiting CTLA-4), independently of PDL-1 status⁵⁵. PDL-1 status was assessed both on archival and fresh tumor material with validated Ventana SP263 immunohistochemistry assay and defining the positivity when 25% or more of tumor cells were positive⁵⁵.

Moreover, Durvalumab reached an overall response rate of 25% in a phase I study of 16 patients with gastric cancer⁵⁶, with a positive correlation between immunohistochemical expression and response.

AstraZeneca and MedImmune, its global biologics research and development arm, announced that the US Food and Drug Administration (FDA) has granted Breakthrough Therapy designation (BTD) for Durvalumab (MEDI4736), an investigational human monoclonal antibody directed against programmed death ligand-1 (PD-L1), for the treatment of patients with PD-L1 positive inoperable or metastatic urothelial bladder cancer whose tumour has progressed during or after one standard platinum-based regimen.

Avelumab (MSB0010718C) is a fully human IgG1 antibody that is thought to promote an antibody-dependent cell-mediated cytotoxicity⁵⁷.

There are at least 16 ongoing studies about Avelumab for the treatment of solid cancers and Hodgkin Lymphoma (www.clinicaltrials.gov), in monotherapy or in association. Recently, Avelumab has been approved for Merkel cell carcinomas by FDA since PDL-1 is expressed in 55% of these tumors.

Moreover, The JAVELIN trial investigated Avelumab in patients with locally advanced or metastatic breast cancer and got a low overall response rate of 4.8%⁵⁸.

By now, there is still no available kit for assessing PDL-1 status already approved and validated, but Merck and Pfizer are collaborating with DAKO for developing a companion diagnostic applicable to Avelumab (Pfizer Pharmaceutical News and Media).

European and Italian state

Concerning Europe, last summer, the European Medicines Agency (EMA) has approved the use of KEYTRUDA (Pembrolizumab) for advanced melanoma in adults because of the results of 3 studies respectively on Phase 1b (KEYNOTE 001)⁵⁹, Phase 2 (KEYNOTE 002)⁶⁰ and Phase 3 (KEYNOTE 006)⁶¹ that have demonstrated significant benefit in survival of patients when compared to ipilimumab (<http://www.ema.europa.eu/ema>).

Moreover, Nivolumab has been approved to treat adults with metastatic squamous non-small cell lung cancer in second line treatment, but recently it is no more refundable from our National Health System (Gazzetta Ufficiale n. 90 of 18/04/2016) and for adult patients with advanced renal cell carcinoma after a previous therapy (<http://www.ema.europa.eu>).

Conclusions

Considering the problematic issues that are still opened about PD-L1 technical assessment and results evaluation/interpretation, further investigations are needed in order to establish standard and reproducible criteria for PD-L1 detection. Maybe further studies should be undertaken to create the clearest “PD-L1 picture” facilitating the pathologist-oncologist “macth” about this matter in order to better select the target neoplastic population bounded for an anti PD1/PD-L1 treatment.

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