Review

PD-L1 Domain Organization, Signaling Motifs and Interactors in Cancer Immunotherapy

David Escors *, Luisa Chocarro, Miriam Echaide, Claudia Rodriguez-Neira, Borja Vilaplana, Grazyna Kochan *

Posted Date: 11 March 2025

doi: 10.20944/preprints202503.0750.v1

Keywords: cancer immunotherapy; B7-H1; immune checkpoint blockade; signal transduction pathways

 doi:10.20944/preprints202503.0750.v1

Article

PD-L1 Domain Organization, Signaling Motifs and Interactors in Cancer Immunotherapy

David Escors *, Luisa Chocarro, Miriam Echaide, Claudia Rodriguez-Neira, Borja Vilaplana and Grazyna Kochan *

OncoImmunology Unit. Navarrabiomed-Fundacion Miguel Servet, Universidad Publica de Navarra (UPNA), Hospital Universitario de Navarra (HUN), Instituto de Investigación Sanitaria de Navarra (IdISNA), 31008, Pamplona, Spain

* Correspondence: descorsm@navarra.es (D.E.); grazyna.kochan@navarra.es (G.K.)

Simple Summary: Immune checkpoint blockade has transformed clinical oncology. However, much is unknown of the cellular and immunological mechanisms behind its success. In recent years, extensive research on the mechanisms of action of programmed death 1 ligand 1 (PD-L1) has uncovered the multiple facets of this immune checkpoint molecule on the biology of the cell. Here we review the current knowledge on PD-L1 structure, signaling motifs and interactors that provide a mechanistic insight into the functions of this key immune checkpoint.

Abstract: Programmed cell death 1 ligand-1 (PD-L1)/ programmed cell death-1 (PD-1) blockade immunotherapies have revolutionized oncology. These therapies consist of blocking the binding of PD-1 expressed in T cells to its ligand PD-L1 expressed by cancer cells using monoclonal antibodies administered systemically. When localized on the surface of cancer cells, PD-L1 delivers inhibitory signals to the T cell through PD-1, inactivating T cell effector functions. In addition to this mechanism of action, PD-L1 also delivers intracellular signals to the cancer cell, in a process termed "reverse signaling" or "intrinsic signaling". This intrinsic signal transduction takes place both in the presence and in the absence of PD-1 binding, and contributes to the survival of cancer cells in response to pro-apoptotic stimuli. PD-L1 intrinsic signaling functions are much wider than originally suspected, and include protection from apoptosis, enhancement of cancer cell proliferation, regulating DNA damage responses and even acting as a co-transcriptional transactivator. The mechanisms governing these functions are currently under intensive research. In this review we provide a perspective on PD-L1 research from distinct points of view, but especially focusing on its structure, signaling motifs and interactors.

Keywords: cancer immunotherapy; B7-H1; immune checkpoint blockade; signal transduction pathways

1. Introduction

Not so long ago, cancer immunotherapies were not considered serious therapeutic options by most oncologists or researchers in oncology. Development of antineoplastic treatments was largely devoted to engineering small molecules and kinase inhibitors targeting cancer cell growth and survival pathways [1–4]. Cancer cells were the targets, and pharmaceutical companies invested significantly into research in cancer cell apoptosis, tumor angiogenesis and processes focusing on the cancer cell. But not centered in the patient's immune system unless in collaboration with academic groups. These strategies were strongly incentivized by the emergence of targeted therapies with small molecule inhibitors specifically targeting mutated variants of well-known oncogenes in cancer cells [5,6]. It could be safely said that immunotherapies were not on their radar.

 doi:10.20944/preprints202503.0750.v1

Nevertheless, it has to be acknowledged that certain immunotherapies were already being given to some patients with limited success, those who did not respond to the standard care of treatments. For example, high-dose interleukin (IL)-2 and interferon (IFNs) therapies [7–11], T cell transfer of tumor-infiltrating T cell (TIL) clones expanded ex vivo, or even autologous T cells genetically modified to express recombinant T cell receptors (TCR) specific for well-known tumor-associated antigens [12–15].

Everything started to change when an anti-cytotoxic T-lymphocyte associated protein (CTLA)-4 antibody produced significant therapeutic efficacies in metastatic melanoma patients [16,17]. But the revolution came in 2012 after the publication of clinical trials evaluating anti-PD-1 and anti-PD-L1 in cancer patients [18,19]. Programmed cell death-1 (PD-1, CD279) was discovered in 1992 in apoptotic T-cells by the research team lead by Tasuku Honjo [20]. A few years later, programmed cell death-1 ligand-1 (PD-L1) was discovered by the team led by Lieping Chen and was named B7 homologue-1 (B7-H1, CD274), a novel member of the B7 family of immunomodulators [21]. PD-L1 turned out to be a negative regulator of immune responses [22–24]. Shortly after its discovery, B7-H1 was identified as the canonical ligand of PD-1 and renamed as PD-L1 [24,25]. Indeed, the PD-1/PD-L1 signaling axis was found to regulate inflammation and systemic tolerance [26–32]. But most importantly, this signaling axis was critical for the evasion of cancer cells from the immune system [23,33–35]. Disruption of PD-1/PD-L1 interactions through several experimental approaches including blockade with monoclonal antibodies targeting either PD-1 or PD-L1, led to an enhancement of anti-cancer immune responses in several tumor models [36–40].

Our team started to work on PD-1/PD-L1 signaling in 2005, and at the time these molecules were just two of many novel potential regulators of anti-cancer immune responses. Indeed, in agreement with the studies from our contemporary investigators, we found that PD-L1 silencing in antigen-presenting dendritic cells (DC) enhanced anti-tumor immunity specially in combination with DC activators [31,41]. In 2012 there was a turning point that took everybody by surprise. The oncology field was shaken after the publication of the first clinical trials with PD-1/PD-L1 blocking antibodies [18,19]. The results were truly unprecedented, with good tolerability and significant therapeutic responses in a wide variety of cancers, surpassing the therapeutic activities already observed with ipilimumab, a clinically approved anti-CTLA-4 antibody [17]. These results confirmed the therapeutic viability of treatments that instead of targeting the cancer cell, were directed towards the immune cell compartment. Within a couple of years, medical oncologists had to be retrained as immunologists, and pharmaceutical companies re-directed investments towards the development of antibodies targeting the so-called “immune checkpoints”[42]. Immunooncology as we understand it today had been born.

2. Programmed Death 1 Ligand 1, PD-L1

PD-L1 is a transmembrane molecule belonging to the B7 family of co-stimulatory molecules. As such, its domain organization is typical to that of the B7 family [21] **(Figure 1)**. PD-L1 is a type I transmembrane surface glycoprotein expressed constitutively by most cells of the myeloid lineage, although its expression can be induced in many cell types following responses to inflammatory stimuli. In cancer cells, PD-L1 expression can also be driven by some oncogenes. PD-L1 presents an immunoglobulin (Ig) variable (V)-like extracellular region, which contains complementary determining-like (CDR) regions constituting the PD-1 binding domain. This interaction is structurally similar to the binding of antigens to antibodies and to the TCR [43,44]. The hydrophobic transmembrane domain is followed by an intracellular region which shows poor sequence similarity to the domains from other B7 counterparts [45]. There is recent evidence suggesting that PD-L1 forms dimers and tetramers in the cell membrane, through association by residues in the transmembrane and intracellular domains [46]. Most importantly, its intracellular domain is critical for reverse signaling in different cell types including cancer cells, and for regulating the molecular stability of the molecule.

 doi:10.20944/preprints202503.0750.v1

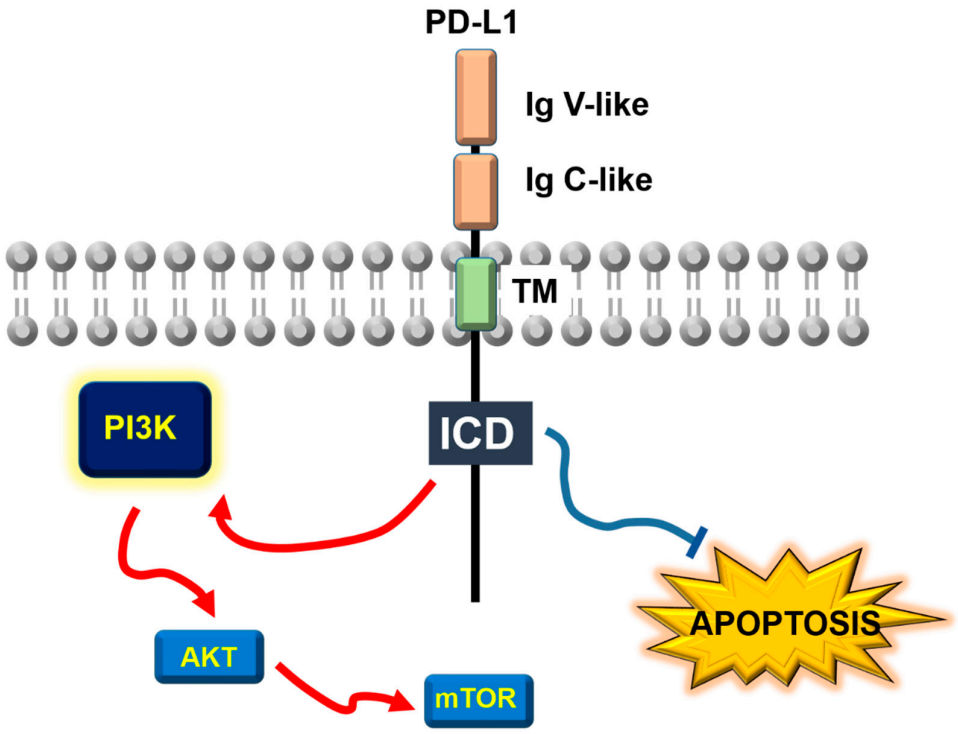


Figure 1. Overall structure and signal transduction functions by PD-L1. Schematic representation of the molecular organization of PD-L1 in the cell membrane. The reverse signaling activating the PI3K/AKT/mTOR pathway and counteracting apoptosis are indicated. Blue line, inhibitory relationship between PD-L1 reverse signaling and induction of apoptotic pathways. Red arrows, activating relationships between the indicated proteins. Ig V and Ig C, indicate immunoglobulin-like variable and conserved sequence motifs. TM, transmembrane domain; ICD, intracellular cytoplasmic domain.

PD-L1 is expressed constitutively in myeloid cells such as DCs, myeloid-derived suppressor cells (MDSCs) and macrophages [29,31,47–51]. Additionally, PD-L1 expression is upregulated in many cell types of different ontologies such as cancer cells, endothelial cells and T cells [27,52–54]. PD-L1 expression can be induced following stimulation with pro-inflammatory cytokines such as interferon gamma (IFNγ), IFN alpha (IFNα), tumor necrosis factor alpha (TNFα), IL-1 or IL-6 through direct or indirect pathways [55–59]. In cancer cells, transcriptional regulation of PD-L1 expression can differ depending on the tumor type or mutational profile in tumor cells [60–62]. In some cases, cancer cells may express PD-L1 constitutively due to the oncogenic activation of signaling pathways such as those controlled by rat sarcoma (RAS), epidermal growth factor receptor (EGFR), mitogen activated protein kinases (MAPK) among others [56,63–68]. PD-L1 expression is also regulated by other mechanisms which include control of its stability and membrane trafficking through ubiquitination, glycosylation, other protein modifications and epigenetic mechanisms [67,69–78].

3. PD-L1 as an Anti-Apoptotic Receptor and AKT/mTOR Regulator

Just before 2012, most of the researchers in PD-1/PD-L1 signaling were focusing on the effects of PD-L1 binding to PD-1, and in the signaling pathways regulated by PD-1 that inhibited T-cells. Thus, among these where the pathways associated to the binding of Src homology region 2 domain-containing phosphatases (SHP) 1 and 2 to ITIM (immunoreceptor tyrosine-based inhibitory) and ITSM (immunoreceptor tyrosine-based switch) motifs in the intracellular domain of PD-1 [79–86], and the up-regulation of E3 ubiquitin ligases of the Casitas B cell lymphoma (CBL) family [31,87–89]. At this point, nobody was paying too much attention to a potential reverse signaling by PD-L1 on the

 doi:10.20944/preprints202503.0750.v1

cancer cell itself, apart from a few studies pioneered by Lieping Chen's team. For several years, the protective role of PD-L1 was restricted to its action as a molecular brake to the effector activities of cancer-specific T cells through its binding with PD-1. In this model, PD-1 signaling into the T-cell was the main protective mechanism of tumors against the T cell attack. In 2004, a screening for T-cell inhibitory molecules expressed by melanoma B16-F10 cancer cells treated with IFNγ uncovered PD-L1 as a protective candidate. Antibody blockade of PD-L1/PD-1 interactions, or absence of PD-1 expression in T cells augmented tumor rejection, suggesting that PD-L1 represented a molecular shield protecting cancer cells from T cell cytotoxicity [34–36]. Surprisingly, in 2008, the Lieping Chen's group provided a molecular protection mechanism for cancer cells driven by PD-L1 independently on its PD-1-engaging activities. In this model, PD-L1 expression conferred cancer cells with resistance to different apoptotic stimuli by transmitting intracellular signals to cancer cells [90] **(Figure 1).** The authors of the study demonstrated that P815 mastocytoma and renal adenocarcinoma Renca cell lines required PD-L1 expression to resist T cell cytotoxicity. As expected, this resistance was abrogated by PD-L1 blocking antibodies. Then, the authors used T cells expressing a PD-1 molecule with its intracellular region replaced by GFP, rendering PD-1 signal-null. In these conditions, cancer cells remained resistant to the T cell attack as long as they expressed PD-L1. This protection was lost when cancer cells expressed a PD-L1 molecule with its intracellular domain replaced by GFP. This study concluded that PD-L1 intracellular signaling conferred resistance to cancer cells against pro-apoptotic stimuli, including first apoptosis signal receptor (Fas)-Fas ligand (FasL) interactions among others [90]. However, when the authors examined major anti-apoptotic and apoptotic pathways, they did not find significant differences between controls versus PD-L1-engaged cancer cells. This study concluded that PD-L1 may be providing an early signal to inhibit apoptotic pathways. Interestingly, the potential signal transduction properties of PD-L1 were described in other experimental contexts. One of such was metabolic competition between cancer cells and T cells. This mechanism is utilized by cancer cells by depleting glucose in the tumor microenvironment. Glucose depletion then interferes with T cell effector activities [91]. A study conducted by Palmer et al. in 2015 demonstrated that treatments with antibodies targeting immune checkpoints CTLA-4, PD-1 and PD-L1 restored glucose concentrations within the tumor microenviroment [92]. These results suggested that immune checkpoint molecules regulated glucose metabolism in mouse sarcoma cells. The authors continued evaluating the effects of PD-L1 blockade or its silencing over glucose metabolism, and demonstrated that PD-L1 blockade in the absence of T cells inhibited the AKR mouse T cell lymphoma (AKT, protein kinase B) / mammalian target of rapamycin (mTOR) pathway **(Figure 1)**. This inhibition led to a reduction in transcription of glycolytic genes, decreasing glucose consumption without affecting cancer cell proliferation or tumor growth. This direct effect of PD-L1 expression over glucose metabolism was later confirmed in 2019 by Kim et al. in lung cancer cell lines [93]. Regulation of mTOR and autophagy pathways by PD-L1 expression in the absence of T cells was also demonstrated by Clark et al. in 2016 in two cancer models: mouse B16 melanoma and ovarian ID8 cells [94]. However in this case, reduction of PD-L1 expression inhibited proliferation of cancer cells, suggesting that these effects might be cell type-specific, as this was also observed in U87 glioblastoma cells [95]. Moreover, the same authors concluded in a follow-up study that PD-L1 signaling played a role in tumor initiating cells (TIC) and their resistance to pro-apoptotic treatments. PD-L1 silencing reduced TIC numbers and their associated functions. But unlike the findings by Chen's team and our own results [90,96], PD-L1 expression in TIC sensitized these cells to IFNγ and rapamycin [97].

A wide range of later studies have confirmed the activating role of PD-L1 intrinsic signaling over phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR pathways, in most instances associated to promoting cancer cell survival and resistance to apoptosis [98–101] **(Figure 1).**

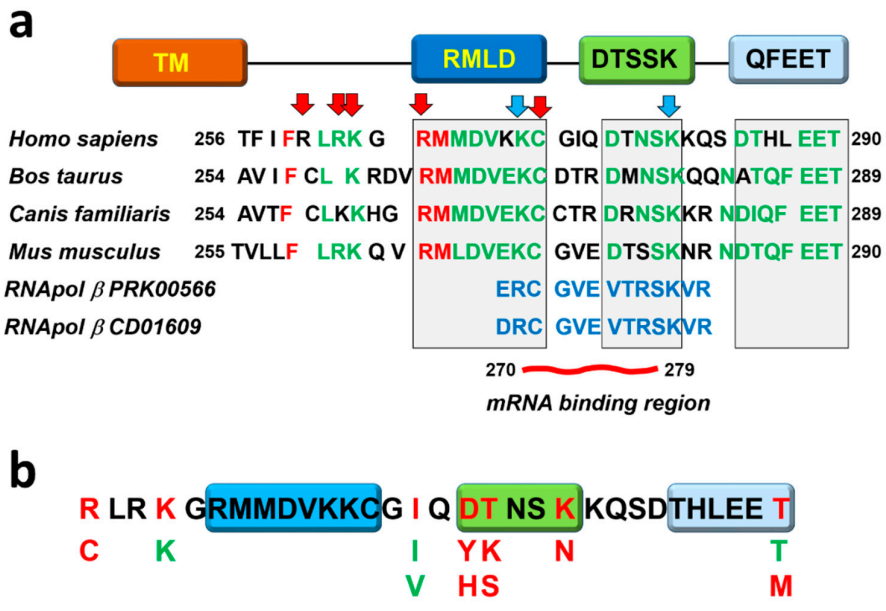
4. PD-L1 Non-Classical Signaling Motifs and Resistance to Interferons in Immunotherapy

In 2017, the research team led by Antoni Ribas published a ground-breaking study demonstrating a mechanism by which some melanoma tumors become resistant to PD-1 blockade

 doi:10.20944/preprints202503.0750.v1

therapies [102]. The authors of the study performed whole-exome sequencing in biopsies from patients with advanced melanoma under PD-1 blockade monotherapy, before and after treatment. Loss-of-function mutations in proteins belonging to the Janus kinase (JAK)1/JAK2 signaling pathways and antigen presentation were identified in non-responders, rendering cancer cells unresponsive to IFNs. The authors concluded that the mechanism of resistance to immunotherapy relied on the abrogation of PD-L1 upregulation following IFN stimulation. The reduced surface expression of PD-L1 was associated to the unresponsiveness to PD-1 blockade due to the absence of PD-L1/PD-1 interactions. These results suggested a close link between inactivation of IFN signaling in cancer cells and resistance to PD-1/PD-L1 blockade immunotherapy [103]. These observations were recently widened to include mutations of mediators of the stimulator of interferon genes (STING) pathway [104]. However, it could be argued that the lack of PD-L1 expression in cancer cells also eliminates the brake to the T-cell whether it expresses PD-1 or not. Hence, T cells could "be free" in principle to exert cytotoxicity towards PD-L1-negative cancer cells. Therefore, additional resistance mechanisms had to exist to explain this apparent paradox.

The molecular relationship between PD-L1 signal transduction and resistance to IFN-induced apoptosis was uncovered after a fortuitous observation in our team. We were engineering living cell-based melanoma vaccines by increasing their immunogenicity through simultaneous secretion of IFNβ and PD-L1 silencing, following an approach that worked for other cytokines [41]. While living melanoma cell lines overproducing IFNβ or with silenced PD-L1 could be derived, it was impossible to keep living melanoma cells with both features [96]. We decided to pursue why this was the case, and found that cells died from IFN-induced apoptosis when PD-L1 expression was reduced. We proved that the critical feature for survival of melanoma cells was the expression of PD-L1. Its silencing, deletion, or blockade with antibodies caused the death of melanoma cells expressing IFNβ. Moreover, the protective activities of PD-L1 were eliminated by truncation of its intracellular region. All results pointed out to PD-L1 possessing intracellular signaling capacities that interfered with IFN-driven apoptosis. Although the research team led by Lieping Chen had delved into this issue and found no specific pathways altered by PD-L1, we followed a classical approach centered on PD-L1 itself [96]. First, we performed multicomparisons of sequences from mammalian PD-L1 molecules. Interestingly, three sequence motifs showed high phylogenetic conservation, "RMLDVEKC", "DTSSK" and "QFEET" as present in the murine PD-L1 sequence **(Figure 2)**.



 doi:10.20944/preprints202503.0750.v1

Figure 2. Conserved motifs in the cytoplasmic domain of PD-L1. (a) On top, transmembrane and intracellular region of PD-L1, with the conserved signaling motifs RMLD(VEKC), DTSSK and QFEET highlighted in boxes. Below, multialignment of selected PD-L1 sequences from the species indicated on the left, or with the indicated bacterial DNA-dependent RNA polymerase ⌀ subunit conserved motifs. Red arrows, arginine and lysine residues involved in PD-L1 stability; Blue arrows, lysine residues with IFN-regulating activities. Residues in red, highly-conserved aminoacids; residues in green, conserved aminoacids; the region binding mRNAs is indicated; Numbers indicate aminoacid positions. (b) Selection of mutations in PD-L1 found in human carcinomas. On top, human PD-L1 sequence including the homologous RMLD(VEKC), DTSSK and QFEET motifs. Below, mutations found in biopsies from human patients. In red, disruptive mutations; in green, conserved mutations.

Using a classical deletion approach, the "RMLDVEKC" motif was absolutely required to inhibit type I and II IFN-driven apoptosis **(Figure 3)**. In contrast, removal of "DTSSK" potentiated the anti-IFN activities of PD-L1, strongly suggesting a function as a negative regulator for this specific motif. The expression of cell surface proteins is frequently regulated by ubiquitination. Hence, the lysine residues in "RMLDVEKC" and "DTSSK" motives were mutated to eliminate this possibility. Their mutations also potentiated the anti-IFN activities of PD-L1, again suggesting that these lysine residues were negative regulators [96], although no apparent changes in PD-L1 cell surface expression were observed in our study. Interestingly, later studies have demonstrated a key role for residue modifications in the carboxy-terminus of PD-L1 that regulate its stability and degradation. For example, arginines R260, R262 and R265, just upstream of the RMLDVEKC motif, were shown to be critical for PD-L1 dimerization and for its complex glycosylation **(Figure 2a)** [46]. Acetylation of K263 regulates its nuclear localization [105,106]. Additionally, R260 and K263 are critical for huntingtin interacting protein 1 related (HIP1R) binding, which targets PD-L1 for degradation [107]. Interestingly, we had previously found mutations within some residues of these motifs selected in skin cancers and other carcinomas [96], highlighting their critical role in carcinogenesis **(Figure 2b)**. Other modifications in the carboxy-terminus have been shown to regulate PD-L1 stabilization. For example palmitoylation in C272 by zinc finger DHHC-type containing 3 (DHHC3) stabilizes PD-L1, allowing cancer cell escape from the T cell attack [108].This palmitoylation inhibits ubiquitination of C272 that causes PD-L1 degradation [109]. Surprisingly, we did not find specific functions for the "QFEET" motif [96].

Overall, IFN signaling pathways constitute a major mechanism for response to PD-1/PD-L1 blockade [45,89,96,110–112]. Hence, it is no wonder that one of the key functions of PD-L1 in cancer cells is to counteract IFN signaling and reduce IFN-induced apoptosis, although the specific pathway may be cancer type-dependent [113]. To shed some light about a possible mechanism, we then studied IFN signal transduction and caspase-dependent regulation of apoptosis in melanoma cells [96] **(Figure 3)**. Melanoma cells responded to IFNβ stimulation by enhancing STAT3 upregulation, but not STAT1 or STAT2. The elimination of PD-L1 selectively induced signal transducer and activator of transcription (STAT)3 phosphorylation in tyrosine 705, but not serine 727 phosphorylation. Abrogation of PD-L1 expression in melanoma cells sensitized them to IFN-driven apoptosis through a pathway dependent on caspases 7 and 9. Overall, our results indicated that the "RMLDVEKC" motif, negatively regulated by the "DTSSK" motif, inhibited STAT3 Y705 phosphorylation and IFN receptor signaling, truncating apoptosis dependent on caspase 7 and 9. Indeed, PD-L1 abrogation in macrophages was shown to potentiate STAT3 activation leading to IL6 production [114]. Something similar was observed in MDSCs [115] .Interestingly, a different mechanism involving phospho-STAT3 was described whereby Y705-STAT3 physically associates with PD-L1, facilitating its nuclear transport and pyroptosis induction in cancer cells through caspase 8 [116]. In other tumor models, the relationship between PD-L1 expression and regulation of JAK-STAT signaling pathways may differ. For example, JAK-STAT-activation has been shown to occur in NSCLC cells caused by PD-L1 reverse signaling in the context of enhancement of angiogenesis, as recently shown by Cavazzoni et el [117].

 doi:10.20944/preprints202503.0750.v1

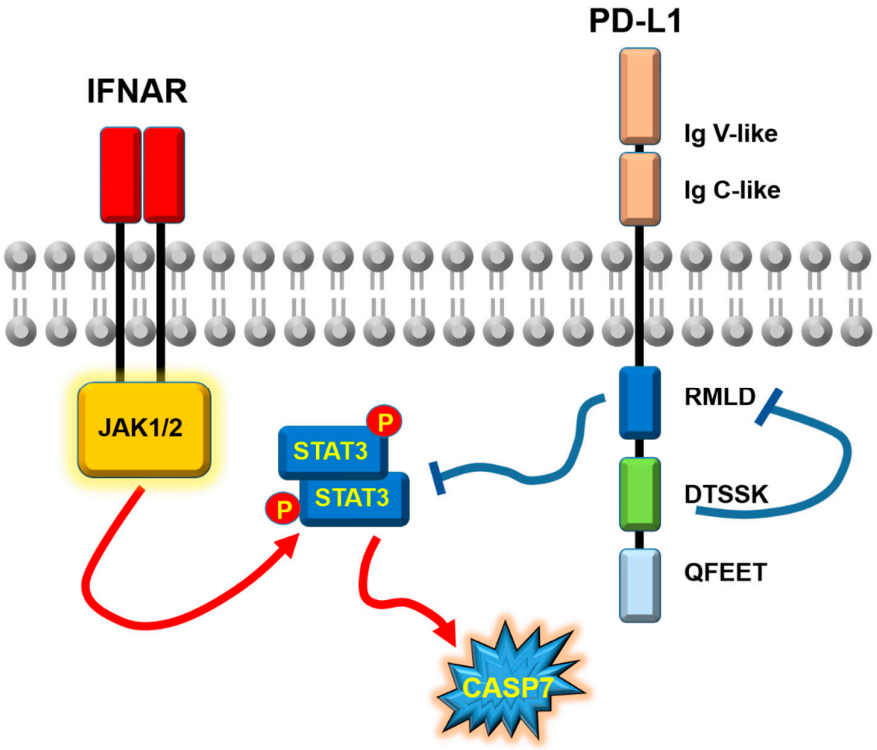


Figure 3. Structure, conserved motifs and anti-IFN activities of PD-L1. Schematic representation of the molecular organization of PD-L1 (right) in the cell membrane, with the main conserved sequence motifs indicated in the figure. The reverse signaling pathway counteracting IFN-driven apoptosis is also depicted. On the left, IFNs signal through JAK1/2 leading to STAT3 phosphorylation and CASP7-dependent apoptosis. Blue lines, inhibitory relationships between the indicated sequence motifs and molecules. Red arrows, activating relationships between the indicated proteins. Ig V and Ig C, indicate immunoglobulin-like variable and conserved sequence motifs. IFNAR, interferon receptor.

Melanoma B16-derived tumors were more sensitive to IFNβ when PD-L1 expression was reduced, a feature dependent on PD-L1 intracellular signal transduction in B16 cells. Indeed, we also found that the human homologue of the "DTSSK" motif was selectively enriched in mutations in several human carcinomas **(Figure 2b)**. These mutations were tested in vitro, demonstrating that they enhanced the anti-IFN capacities of human PD-L1 [96]. Importantly, antibody blockade of PD-L1 decreased cancer cell proliferation and enhanced sensitivity to IFNβ in murine and human melanoma cancer cells [96], a mechanism later confirmed in other tumor types [118,119]. The role of PD-L1 as a factor favoring cancer cell proliferation is also supported by studies in other tumor models, in which agonistic PD-L1 antibodies induce cell growth and survival, such as in Hodgkin lymphoma [120]. Thus, apart from inactivating mutations of the IFN signal transduction pathway and antigen presentation, other mutations directly in PD-L1 can be selected in human carcinomas that protect cancer cells from IFN-induced apoptosis.

5. The PD-L1 Interactome

We continued with a follow-up study taking advantage that the human interactome had been published [121]. These data was obtained by mass spectrometry analyses in human embryonic kidney (HEK)-293T cells after antibody-based affinity purification of targets of interest bound to their interacting proteins. An interactome associated to human PD-L1 was constructed for the first time with proteins associated to affinity-purified PD-L1 [45]. This interactome can be divided in three

 doi:10.20944/preprints202503.0750.v1

functional groups: (1) DNA-damage response; (2) nuclear protein and RNA import/export group; (3) and Golgi- endoplasmic reticulum (ER) interactors **(Figure 4).**

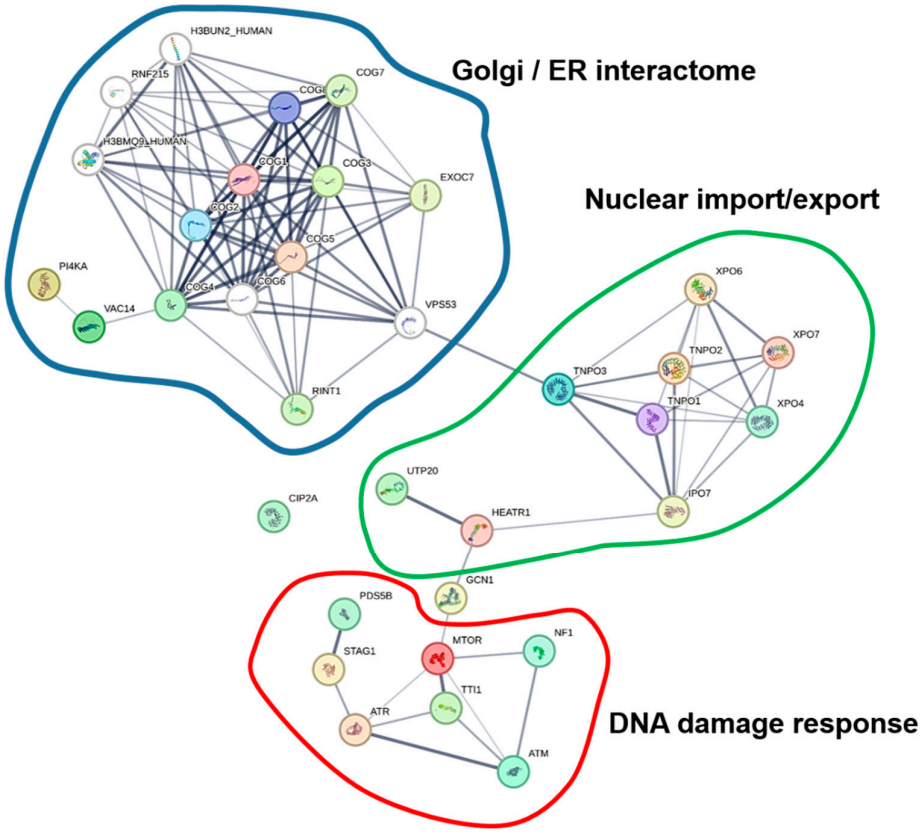


Figure 4. Interactome associated to human PD-L1. Interactors detected experimentally in [121] are shown and arranged reconstructing their interactions with STRING (https://string-db.org/) [45]. Predicted nodes are shown in white. Interactomes have been grouped in three main classes according to their associated functions, as indicated in the figure.

The first group of proteins included mTOR, several kinases that regulate ancho-independent cell growth, and surprisingly, key regulators of the DNA damage response pathway including ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related protein) **(Figure 4)** [45]. It had already been experimentally demonstrated at the time that PD-L1 was regulating signaling through mTOR/AKT pathways [99], as extensively described above. However, the association of ATM and ATR proteins to PD-L1 was unexpected, strongly suggesting a direct link between PD-L1 and regulation of the DNA damage response pathway [45]. Indeed, radiotherapy had been found to induce PD-L1 upregulation in cancer cells through the activity of ATM/ATR [122]. Later on in 2021, Ozawa et al. showed that IFN-mediated PD-L1 upregulation was associated to the double-strand breaks (DSB) repair pathway in patients with colitic cancer, mainly by immunofluorescence microscopy techniques [123]. Indeed, PD-L1 upregulation was shown to be associated to resistance of cancer cells to radiotherapy in a genome-wide CRISPR/Cas9-based screening, by accelerating DBS repair. Interestingly, this mechanism was dependent on PD-L1 N219 deglycosylation and its translocation to the cell nucleus. PD-L1 translocation was mediated by CMTM6, one of PD-L1's known interactors [69,70] **(Figure 5)**. In the nucleus, PD-L1 was shown to bind KU (X-Ray repair cross complementing 6 and 5 dimer, XRCC6/XRCC5) through its IgC-like domain and facilitate non-homologous end joining recombination (NHEJ) [124]. Further research supported that nuclear PD-L1 was not an artefact as suggested by other authors [125], and confirmed that PD-L1 can translocate to the nucleus [106,124,126,127]. Hou et al. showed in a study that PD-L1

 doi:10.20944/preprints202503.0750.v1

translocated to the nucleus through an interaction of its carboxy terminus with Y705-phosphorylated STAT3, where PD-L1/STAT3 regulated gasdermin C (GSDMC) transcription in cancer cells in response to hypoxia [116] **(Figure 5)**. Similar links between PD-L1 and DNA repair mechanisms have been also published by other authors [128,129]. Very recently, a direct association between nuclear PD-L1 and ATR which regulates DNA damage response was demonstrated [130] **(Figure 5)**. A role for regulation of DNA repair mechanisms through binding to BRCA1 associated RING domain 1 (BARD1) was also described in several cancer types [128]. The authors of this study suggested that PD-L1 may be promoting BARD1 translocation into the nucleus, favouring breast cancer 1 (BRCA1) nuclear foci formation and homologous recombination. However, the authors of this study did not map the potential binding site for BARD1.

The second group of interactors that we originally described were associated to mRNA import/export and protein nuclear import/export pathways **(Figure 4)** [45]. At that time, we did not consider these results to be relevant, but since then, many research teams have demonstrated the nuclear import/export of PD-L1 associated to glycosylation and deglycosylation pathways, binding to phosphorylated STAT-3, CKLF like MARVEL transmembrane domain containing 6 (CMTM6) binding or by other mechanisms **(Figure 5)**. As mentioned above, it turned out that nuclear PD-L1 is related to key and very relevant functions in cancer cell biology, inflammation and immunity [54,106,123,124,126–128,130].

The third group of interactors included Golgi and ER proteins, as would be expected for a transmembrane glycoprotein. Indeed, the regulation of PD-L1 transport, ubiquitination and degradation turned out to be a major immunoregulatory mechanism in tumor biology [31,69–71,73–78,124,126,131–133].

In 2021, a PD-L1-associated metabolic interactome was also obtained and its relationship with choline kinase (CHK)α was established by analysing breast cancer (MDA-MB-231 and SUM-149) and pancreatic ductal adenocarcinoma (Pa09C and Pa20C) cell lines [134]. The authors used high resolution magnetic resonance spectroscopy, following PD-L1 and CHKα silencing with small-interfering RNAs. An inverse correlation in expression between both proteins was demonstrated. Inverse changes in metabolites were identified regulated by both proteins, as well as lipid profiles, which were mediated through CHKα, cyclooxygenase 2 (COX2) and transforming growth factor beta (TGFβ).

A later study by Nieto et al. defined PD-L1's intracellular interactome and the role of its carboxy-terminus in mediating signal transduction in a head and neck squamous cell carcinoma model [135]. Interestingly, there were some differences between the interactome in the absence and presence of PD-1 binding to PD-L1. The authors of the study also demonstrated a physical association between PD-L1, interleukin enhancer binding factor (ILF)2, ILF3, family with sequence similarity 129 member B (FAM129B) and protein tyrosine phosphatase non-receptor type 1 (PTPN1) by co-immunoprecipitation **(Figure 5)**. Interestingly, ILF2 and ILF3 associated to PD-L1 in the absence and in the presence of PD-1 interaction. In this experimental model, ILF2-ILF3 binding to PD-L1 preceded and induced STAT3 phosphorylation, favoring cancer cell progression.

 doi:10.20944/preprints202503.0750.v1

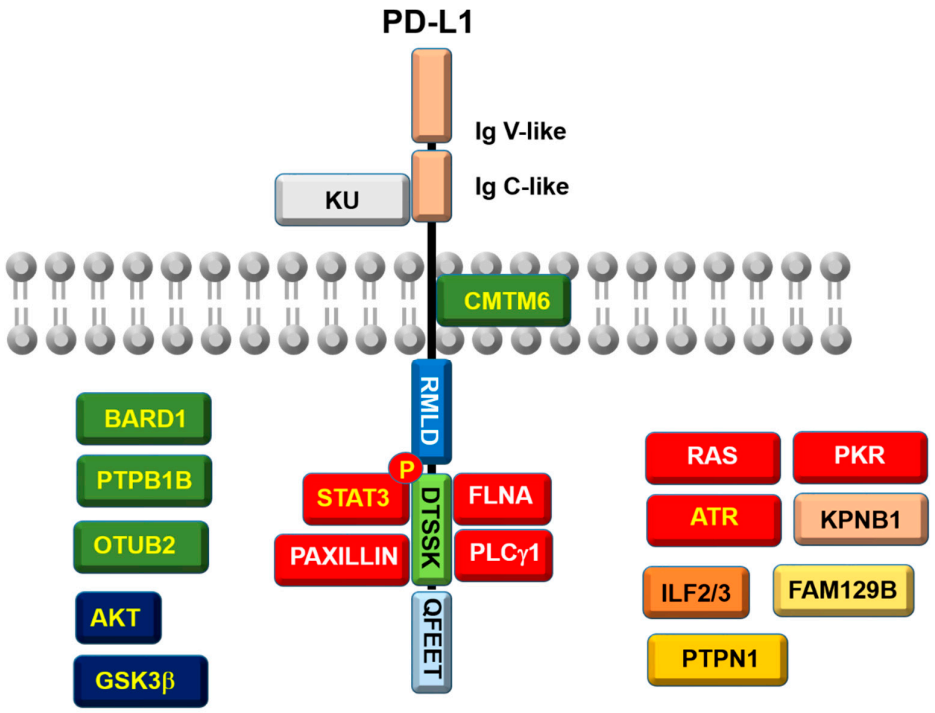


Figure 5. PD-L1 interacting proteins. In the figure, the PD-L1 structure is represented with its conserved sequence motifs as indicated. Experimentally identified binding partners other than the originally published [45] are indicated in the figure. Those in close contact correspond to interactors for which the interacting domain in PD-L1 is known, as schematically shown in the figure. *Interactome associated to human PD-L1.*

Other direct interactors identified to date include glycogen synthase kinase 3 beta (GSK3β) in head and neck squamous carcinoma [136] **(Figure 5)**. Upregulated PD-L1 following irradiation in radioresistant cancer cells enhanced its association with GSK3β, leading to its inactivation by phosphorylation and subsequent increase in cell proliferation and survival. AKT was also found to be a direct interactor at least by coimmunoprecipitation with PD-L1 in platelets, sustaining its activation [100] **(Figure 5)**.

In triple-negative breast cancer cells, PD-L1 was shown to co-immunoprecipitate with the tyrosine phosphatase PTP1B through PD-L1's carboxy terminus **(Figure 5)**. However, the specific motifs mediating this binding were not identified in this study. The authors of the study proposed a mechanism by which PD-L1 binding to PTPB1B prevented GSK3β-mediated phosphorylation and subsequent degradation by ubiquitination of Snail, promoting epithelial to mesenchymal transition (EMT) in cancer cells [137].

The deubiquitinating enzyme OTU deubiquitinase ubiquitin aldehyde binding 2 (OTUB2) was also shown to be a direct interactor with cytoplasmic PD-L1 in many cancer cell types [138] . Interestingly, this interactor, at least its catalytically active form, was shown to bind to PD-L1's intracellular domain both in vivo and also in vitro with purified proteins **(Figure 5)**. However, the specific motif mediating this interaction was not mapped. The authors of this study proposed that catalytically active OTUB2 binds to PD-L1 and prevents its degradation.

Intriguingly, karyopherin subunit beta 1 (KPNB1) has been shown to associate to PD-L1 and mediate its translocation to the nucleus, where it cooperates with specificity protein 1 (SP1) to transactivate growth arrest specific 6 (GAS6) transcription, leading to enhanced non-small cell lung cancer (NSCLC) proliferation [127] **(Figure 5)**. Likewise, nuclear PD-L1 has been shown to associate to epidermal growth factor receptor 1 (EGR1) promoter, leading to its transcription by association

 doi:10.20944/preprints202503.0750.v1

with phospho-STAT3 [106]. These results extend PD-L1's role to that of transcriptional co-activator, if not a transcription factor by itself.

6. The DNA-Dependent RNA Polymerase-Like Domain

A surprising result from our original bioinformatic analyses on PD-L1 was the identification of a conserved sequence in the intracytoplasmic domain corresponding to a highly conserved bacterial DNA-dependent RNA polymerase β subunit motif **(Figure 2b)** [45,96]. This result was completely unexpected. The RNApol motif encompassed part of the RMLD and the complete DTSSK sequences **(Figure 2b)**. We demonstrated that these sequences contributed to the anti-IFN response, but the relevance of an RNApol motif in PD-L1's carboxy terminus was unclear at the time. However, in 2019 Tu et al. demonstrated that PD-L1 could act as an RNA-binding protein [139], mapping the interacting domain (aminoacids 270-279) to most of the RNApol-like motif **(Figure 2b)**. The authors of this study showed that intracellular PD-L1 stabilized several mRNAs including those coding for nibrin (NBS1) and BRCA1 in HCT116 and MDA-MD-231 cell lines. PD-L1 protected mRNAs from degradation and increased resistance to DNA damage. It is tempting to speculate that the RNApol-like motif contributes to DNA/RNA binding and participate in transcriptional regulation and DNA repair as already shown [69,70,116,124,127–130].

7. Other Pathways Regulated by PD-L1 Reverse Signaling

Since the early publications on PD-L1 intrinsic signaling in cancer cells, subsequent research has reinforced its critical role in a plethora of biological regulatory mechanisms. Theivanthiran et al. highlighted the role played by PD-L1 associated to the inflammasome to drive granulocytic MDSC recruitment into the tumor, which caused resistance to PD-1 blockade immunotherapy [140]. Indeed, anti-PD-L1 antibody plus IFN treatment of BRAFV600E PTEN-/- melanoma cells was shown to activate NOD-like receptor protein 3 (NLRP3) through protein kinase R (PKR) binding **(Figure 5)**. This effect was shown to take place in LLC mouse lung adenocarcinoma cells. This mechanism was driven by PD-L1 crosslinking and STAT3 suppression, which seems to be a central common mechanism in PD-L1 intrinsic signaling in several cancer cell models [96,140].

Intrinsic PD-L1 reverse signaling is not restricted to cancer cells or exclusively linked to immune escape. Lucas et al. demonstrated a critical role for PD-L1 reverse signaling in regulating the migration of conventional activated DCs from the skin to the draining lymph node [141]. This mechanism was shown to be required for proper T cell priming, and relied on activation of c-c chemokine receptor type 7 (CCR7) signaling through the DTSSK domain of PD-L1. Most importantly, the DTSSK domain in PD-L1 was found also critical in lymphatic endothelial cells (LECs) for lymphatic remodelling following immune stimulation with polyIC, TNFα and IFNβ [142]. Inactivating mutations in the DTSSK domain by replacing the TSS motif with alanines impeded actin polymerization and LEC movement. Interestingly, these mutations reduced the binding of PD-L1 to paxillin and phosphorylated S727 and Y705 STAT3, required for protein assembly at focal adhesions **(Figure 5)**. In this study, the authors further characterized PD-L1 interactors and phospho-interactors after immunoprecipitation, finding 5 differential proteins between wild-type PD-L1 and DTSSK-mutated PD-L1 [142]. Binding of PD-L1 to H-RAS was shown by co-immunoprecipitation in 293T and LN229 cells by Qiu et al., although the specific domain regulating this interaction was not described in their study [143] **(Figure 5)**. The authors showed that PD-L1 promoted RAS activation leading to EMT in glioblastoma multiforme. A similar mechanism was observed in colorectal cancer cells, regulated through ERK/AKT signal transduction pathway after binding to KRAS, as shown by co-immunoprecipitation [101]. In this study, PD-L1/PD-L1 interactions were mediated extracellular signal-mediated kinase (ERK)/AKT activation, although the specific domain of PD-L1 associated to KRAS was not identified **(Figure 5)**. EMT was recently shown to be induced by PD-L1 intrinsic signaling, but in this study a MAPK p38-dependent mechanism was involved [144]. Hence, PD-L1 expression activated the MAPK ERK pathway, accelerating cancer cell growth and tumor

 doi:10.20944/preprints202503.0750.v1

progression. STAT3-dependent signaling in CD4 T cells was also triggered by PD-L1 binding, and induced an anergic phenotype in CD8 T cells and a regulatory phenotype in CD4 T cells [145,146].

PD-L1 has been shown to directly bind phospholipase C in an EGFR-dependent manner in several lung cancer cell variants, regulating Rho GTPases, which enhanced tumorigenicity [119]. PD-L1 interactors were probed in a yeast 2-hybrid assay, finding filamin A (FLNA) and phospholipase C gamma 1 (PLCγ1). The authors of this study concluded that PD-L1 associated with PLC which enhanced its transphophorylation by EGFRs, and mapped the interacting domain at the region containing the DTSSK motif **(Figure 5).**

8. Conclusions

The clinical use of PD-L1/PD-1 blockade immunotherapies came fast and developed faster. Much faster than research into the molecular and cellular mechanisms of PD-1 and PD-L1 functions. Nevertheless, the extraordinary success of these therapies ignited research into the functions of these immune checkpoint inhibitors. During the last 10 years, we have discovered multiple facets of PD-L1 in cell biology. Not only as the so-called T-cell break in immunotherapy, but also as a molecule actively participating in cell proliferation, survival and in DNA damage responses. The importance of nuclear PD-L1 has also arisen recently, suggesting that PD-L1 can function as a co-transcriptional activator as well. The knowledge acquired on its structural organization, functions and interactors will help us in the identification of novel targets, and design improved therapies that can be used in combination with standard immune checkpoint blockade immunotherapies.

Author Contributions: All authors contributed to the writing of the review. DE and GK chose the theme and wrote the first draft.

Funding: The OncoImmunology group is funded by grants from I+D Strategic Projects, Departamento de Industria, Gobierno de Navarra, ARNMUNE (0011-1411-2023-000111) and BIOOLE-UP (PC24-BIOOLE-UP-005-007-003); Biomedicine projects from Departamento de Salud, Gobierno de Navarra, BMED 036/23; Instituto de Salud Carlos III, FIS Project (PI23/00196); European Project Horizon 2020-SC1-BHC-2018-2020, ISOLDA (Ref ID: 848166); LC is financed by Instituto de Salud Carlos III, co-financed by FEDER funds, "Contratos PFIS: contratos predoctorales de formación en investigación en salud" (FI21/00080); ME is financed by the Navarrabiomed-Fundación Miguel Servet predoctoral contract.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No data was generated.

Acknowledgments: We sincerely acknowledge all the patients and families that have generously participated in the investigations by the OncoImmunology group, and also to the nursing staff of the Medical Oncology Day Care at University Hospital of Navarre.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Nefedova, Y.; Sullivan, D.M.; Bolick, S.C.; Dalton, W.S.; Gabrilovich, D.I. Inhibition of Notch signaling induces apoptosis of myeloma cells and enhances sensitivity to chemotherapy. *Blood* **2008**, *111*, 2220-2229.
2. Nishioka, C.; Ikezoe, T.; Yang, J.; Takeshita, A.; Taniguchi, A.; Komatsu, N.; Togitani, K.; Koeffler, H.P.; Yokoyama, A. Blockade of MEK/ERK signaling enhances sunitinib-induced growth inhibition and apoptosis of leukemia cells possessing activating mutations of the FLT3 gene. *Leuk Res* **2008**, *32*, 865-872.
3. Xin, H.; Zhang, C.; Herrmann, A.; Du, Y.; Figlin, R.; Yu, H. Sunitinib inhibition of Stat3 induces renal cell carcinoma tumor cell apoptosis and reduces immunosuppressive cells. *Cancer research* **2009**, *69*, 2506-2513.

 doi:10.20944/preprints202503.0750.v1

4. Flaherty, K.T.; Robert, C.; Hersey, P.; Nathan, P.; Garbe, C.; Milhem, M.; Demidov, L.V.; Hassel, J.C.; Rutkowski, P.; Mohr, P., et al. Improved survival with MEK inhibition in BRAF-mutated melanoma. *The New England journal of medicine* **2012**, *367*, 107-114.
5. Johnson, D.B.; Sosman, J.A. Update on the targeted therapy of melanoma. *Curr Treat Options Oncol* **2013**, *14*, 280-292, doi:10.1007/s11864-013-0226-8.
6. Arozarena, I.; Wellbrock, C. Overcoming resistance to BRAF inhibitors. *Annals of translational medicine* **2017**, *5*, 387, doi:10.21037/atm.2017.06.09.
7. Martin, A.; Nerenstone, S.; Urba, W.J.; Longo, D.L.; Lawrence, J.B.; Clark, J.W.; Hawkins, M.J.; Creekmore, S.P.; Smith, J.W., 2nd; Steis, R.G. Treatment of hairy cell leukemia with alternating cycles of pentostatin and recombinant leukocyte A interferon: results of a phase II study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **1990**, *8*, 721-730.
8. Dunn, G.P.; Bruce, A.T.; Sheehan, K.C.; Shankaran, V.; Uppaluri, R.; Bui, J.D.; Diamond, M.S.; Koebel, C.M.; Arthur, C.; White, J.M., et al. A critical function for type I interferons in cancer immunoediting. *Nature immunology* **2005**, *6*, 722-729, doi:10.1038/ni1213.
9. Smyth, M.J. Type I interferon and cancer immunoediting. *Nature immunology* **2005**, *6*, 646-648, doi:10.1038/ni0705-646.
10. Dunn, G.P.; Koebel, C.M.; Schreiber, R.D. Interferons, immunity and cancer immunoediting. *Nature reviews. Immunology* **2006**, *6*, 836-848, doi:10.1038/nri1961.
11. Qu, J.; Zhao, M.; Teng, Y.; Zhang, Y.; Hou, K.; Jiang, Y.; Yang, X.; Shang, H.; Qu, X.; Liu, Y. Interferon-alpha sensitizes human gastric cancer cells to TRAIL-induced apoptosis via activation of the c-CBL-dependent MAPK/ERK pathway. *Cancer biology & therapy* **2011**, *12*, 494-502, doi:10.4161/cbt.12.6.15973.
12. Rosenberg, S.A.; Mule, J.J.; Spiess, P.J.; Reichert, C.M.; Schwarz, S.L. Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2. *The Journal of experimental medicine* **1985**, *161*, 1169-1188, doi:10.1084/jem.161.5.1169.
13. Coccoris, M.; Straetemans, T.; Govers, C.; Lamers, C.; Sleijfer, S.; Debets, R. T cell receptor (TCR) gene therapy to treat melanoma: lessons from clinical and preclinical studies. *Expert opinion on biological therapy* **2010**, *10*, 547-562.
14. Johnson, L.A.; Heemskerk, B.; Powell, D.J.J.; Cohen, C.J.; Morgan, R.A.; Dudley, M.E.; Robbins, P.F.; Rosenberg, S.A. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. *Journal of immunology* **2006**, *177*, 6548-6559.
15. Escors, D. Tumour immunogenicity, antigen presentation and immunological barriers in cancer immunotherapy. *New J Sci* **2014**, *2014*, doi:10.1155/2014/734515.
16. Hodi, F.S.; Oble, D.A.; Drappatz, J.; Velazquez, E.F.; Ramaiya, N.; Ramakrishna, N.; Day, A.L.; Kruse, A.; Mac Rae, S.; Hoos, A., et al. CTLA-4 blockade with ipilimumab induces significant clinical benefit in a female with melanoma metastases to the CNS. *Nat Clin Pract Oncol* **2008**, *5*, 557-561, doi:10.1038/ncponc1183
1. ncponc1183 [pii].
17. Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C., et al. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England journal of medicine* **2010**, *363*, 711-723, doi:10.1056/NEJMoa1003466
2. NEJMoa1003466 [pii].
18. Brahmer, J.R.; Tykodi, S.S.; Chow, L.Q.; Hwu, W.J.; Topalian, S.L.; Hwu, P.; Drake, C.G.; Camacho, L.H.; Kauh, J.; Odunsi, K., et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *The New England journal of medicine* **2012**, *366*, 2455-2465.
19. Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B., et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *The New England journal of medicine* **2012**, *366*, 2443-2454.
20. Ishida, Y.; Agata, Y.; Shibahara, K.; Honjo, T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* **1992**, *11*, 3887-3895.
21. Greenwald, R.J.; Freeman, G.J.; Sharpe, A.H. The B7 family revisited. *Annu Rev Immunol* **2005**, *23*, 515-548, doi:10.1146/annurev.immunol.23.021704.115611.

 doi:10.20944/preprints202503.0750.v1

22. Dong, H.; Zhu, G.; Tamada, K.; Chen, L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nature medicine* **1999**, *5*, 1365-1369.
23. Dong, H.; Strome, S.E.; Salomao, D.R.; Tamura, H.; Hirano, F.; Flies, D.B.; Roche, P.C.; Lu, J.; Zhu, G.; Tamada, K., et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nature medicine* **2002**, *8*, 793-800, doi:10.1038/nm730.
24. Freeman, G.J.; Long, A.J.; Iwai, Y.; Bourque, K.; Chernova, T.; Nishimura, H.; Fitz, L.J.; Malenkovich, N.; Okazaki, T.; Byrne, M.C., et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *The Journal of experimental medicine* **2000**, *192*, 1027-1034.
25. Butte, M.J.; Keir, M.E.; Phamduy, T.B.; Sharpe, A.H.; Freeman, G.J. Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses. *Immunity* **2007**, *27*, 111-122.
26. Kanai, T.; Totsuka, T.; Uraushihara, K.; Makita, S.; Nakamura, T.; Koganei, K.; Fukushima, T.; Akiba, H.; Yagita, H.; Okumura, K., et al. Blockade of B7-H1 suppresses the development of chronic intestinal inflammation. *Journal of immunology* **2003**, *171*, 4156-4163.
27. Mirza, N.; Duque, M.A.; Dominguez, A.L.; Schrum, A.G.; Dong, H.; Lustgarten, J. B7-H1 expression on old CD8+ T cells negatively regulates the activation of immune responses in aged animals. *Journal of immunology* **2010**, *184*, 5466-5474.
28. Wang, L.; Pino-Lagos, K.; de Vries, V.C.; Guleria, I.; Sayegh, M.H.; Noelle, R.J. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105*, 9331-9336, doi:0710441105 [pii]
3. 10.1073/pnas.0710441105.
29. Latchman, Y.E.; Liang, S.C.; Wu, Y.; Chernova, T.; Sobel, R.A.; Klemm, M.; Kuchroo, V.K.; Freeman, G.J.; Sharpe, A.H. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *Proceedings of the National Academy of Sciences of the United States of America* **2004**, *101*, 10691-10696.
30. Fife, B.T.; Pauken, K.E.; Eagar, T.N.; Obu, T.; Wu, J.; Tang, Q.; Azuma, M.; Krummel, M.F.; Bluestone, J.A. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. *Nature immunology* **2009**, *10*, 1185-1192, doi:ni.1790 [pii]
4. 10.1038/ni.1790.
31. Karwacz, K.; Bricogne, C.; Macdonald, D.; Arce, F.; Bennett, C.L.; Collins, M.; Escors, D. PD-L1 co-stimulation contributes to ligand-induced T cell receptor down-modulation on CD8(+) T cells. *EMBO molecular medicine* **2011**, *3*, 581-592, doi:10.1002/emmm.201100165.
32. Keir, M.E.; Butte, M.J.; Freeman, G.J.; Sharpe, A.H. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* **2008**, *26*, 677-704, doi:10.1146/annurev.immunol.26.021607.090331.
33. Zhang, L.; Gajewski, T.F.; Kline, J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. *Blood* **2009**, *114*, 1545-1552.
34. Blank, C.; Brown, I.; Peterson, A.C.; Spiotto, M.; Iwai, Y.; Honjo, T.; Gajewski, T.F. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. *Cancer research* **2004**, *64*, 1140-1145.
35. Blank, C.; Gajewski, T.F.; Mackensen, A. Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy. *Cancer immunology, immunotherapy : CII* **2005**, *54*, 307-314, doi:10.1007/s00262-004-0593-x.
36. Hirano, F.; Kaneko, K.; Tamura, H.; Dong, H.; Wang, S.; Ichikawa, M.; Rietz, C.; Flies, D.B.; Lau, J.S.; Zhu, G., et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer research* **2005**, *65*, 1089-1096.
37. Okudaira, K.; Hokari, R.; Tsuzuki, Y.; Okada, Y.; Komoto, S.; Watanabe, C.; Kurihara, C.; Kawaguchi, A.; Nagao, S.; Azuma, M., et al. Blockade of B7-H1 or B7-DC induces an anti-tumor effect in a mouse pancreatic cancer model. *International journal of oncology* **2009**, *35*, 741-749.
38. Curran, M.A.; Montalvo, W.; Yagita, H.; Allison, J.P. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proceedings of the National Academy of Sciences of the United States of America* **2010**, *107*, 4275-4280.

 doi:10.20944/preprints202503.0750.v1

39. Dulos, J.; Carven, G.J.; van Boxtel, S.J.; Evers, S.; Driessen-Engels, L.J.; Hobo, W.; Gorecka, M.A.; de Haan, A.F.; Mulders, P.; Punt, C.J., et al. PD-1 blockade augments Th1 and Th17 and suppresses Th2 responses in peripheral blood from patients with prostate and advanced melanoma cancer. *Journal of immunotherapy* **2012**, *35*, 169-178.
40. Pardoll, D.M. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* **2012**, *12*, 252-264, doi:10.1038/nrc3239.
41. Liechtenstein, T.; Perez-Janices, N.; Blanco-Luquin, I.; Schwarze, J.; Dufait, I.; Lanna, A.; De Ridder, M.; Guerrero-Setas, D.; Breckpot, K.; Escors, D. Anti-melanoma vaccines engineered to simultaneously modulate cytokine priming and silence PD-L1 characterized using ex vivo myeloid-derived suppressor cells as a readout of therapeutic efficacy. *Oncoimmunology* **2014**, *3*, e29178.
42. Weber, J. Immune checkpoint proteins: a new therapeutic paradigm for cancer--preclinical background: CTLA-4 and PD-1 blockade. *Seminars in oncology* **2010**, *37*, 430-439, doi:10.1053/j.seminoncol.2010.09.005.
43. Zak, K.M.; Kitel, R.; Przetocka, S.; Golik, P.; Guzik, K.; Musielak, B.; Domling, A.; Dubin, G.; Holak, T.A. Structure of the Complex of Human Programmed Death 1, PD-1, and Its Ligand PD-L1. *Structure* **2015**, *23*, 2341-2348, doi:10.1016/j.str.2015.09.010.
44. Lin, D.Y.; Tanaka, Y.; Iwasaki, M.; Gittis, A.G.; Su, H.P.; Mikami, B.; Okazaki, T.; Honjo, T.; Minato, N.; Garboczi, D.N. The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors. *Proceedings of the National Academy of Sciences of the United States of America* **2008**, *105*, 3011-3016.
45. Escors, D.; Gato-Canas, M.; Zuazo, M.; Arasanz, H.; Garcia-Granda, M.J.; Vera, R.; Kochan, G. The intracellular signalosome of PD-L1 in cancer cells. *Signal transduction and targeted therapy* **2018**, *3*, 26, doi:10.1038/s41392-018-0022-9.
46. Zhou, L.; Chai, F.; He, Y.; Zhou, Z.; Guo, S.; Li, P.; Sun, Q.; Zu, X.; Liu, X.; Huang, Q., et al. Homodimerized cytoplasmic domain of PD-L1 regulates its complex glycosylation in living cells. *Communications biology* **2022**, *5*, 887, doi:10.1038/s42003-022-03845-4.
47. Gato-Canas, M.; Martinez de Morentin, X.; Blanco-Luquin, I.; Fernandez-Irigoyen, J.; Zudaire, I.; Liechtenstein, T.; Arasanz, H.; Lozano, T.; Casares, N.; Chaikuad, A., et al. A core of kinase-regulated interactomes defines the neoplastic MDSC lineage. *Oncotarget* **2015**, *6*, 27160-27175, doi:10.18632/oncotarget.4746.
48. Escors, D.; Liechtenstein, T.; Perez-Janices, N.; Schwarze, J.; Dufait, I.; Goyvaerts, C.; Lanna, A.; Arce, F.; Blanco-Luquin, I.; Kochan, G., et al. Assessing T-cell responses in anticancer immunotherapy: Dendritic cells or myeloid-derived suppressor cells? *Oncoimmunology* **2013**, *2*, e26148, doi:10.4161/onci.26148.
49. Akbari, O.; Freeman, G.J.; Meyer, E.H.; Greenfield, E.A.; Chang, T.T.; Sharpe, A.H.; Berry, G.; DeKruyff, R.H.; Umetsu, D.T. Antigen-specific regulatory T cells develop via the ICOS-ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. *Nature medicine* **2002**, *8*, 1024-1032.
50. Blanco, E.; Silva-Pilipich, N.; Bocanegra, A.; Chocarro, L.; Procopio, A.; Ausin, K.; Fernandez-Irigoyen, J.; Fernandez, L.; Razquin, N.; Igea, A., et al. Oleuropein-driven reprogramming of the myeloid cell compartment to sensitise tumours to PD-1/PD-L1 blockade strategies. *British journal of cancer* **2024**, 10.1038/s41416-023-02561-y, doi:10.1038/s41416-023-02561-y.
51. Lu, C.; Redd, P.S.; Lee, J.R.; Savage, N.; Liu, K. The expression profiles and regulation of PD-L1 in tumor-induced myeloid-derived suppressor cells. *Oncoimmunology* **2016**, *5*, e1247135, doi:10.1080/2162402X.2016.1247135.
52. Kozako, T.; Yoshimitsu, M.; Fujiwara, H.; Masamoto, I.; Horai, S.; White, Y.; Akimoto, M.; Suzuki, S.; Matsushita, K.; Uozumi, K., et al. PD-1/PD-L1 expression in human T-cell leukemia virus type 1 carriers and adult T-cell leukemia/lymphoma patients. *Leukemia* **2009**, *23*, 375-382.
53. Yang, W.; Li, H.; Chen, P.W.; Alizadeh, H.; He, Y.; Hogan, R.N.; Niederkorn, J.Y. PD-L1 expression on human ocular cells and its possible role in regulating immune-mediated ocular inflammation. *Invest Ophthalmol Vis Sci* **2009**, *50*, 273-280, doi:10.1167/iovs.08-2397.
54. Su, N.W.; Dai, S.H.; Hsu, K.; Chang, K.M.; Ko, C.C.; Kao, C.W.; Chang, Y.F.; Chen, C.G. PD-L1-positive circulating endothelial progenitor cells associated with immune response to PD-1 blockade in patients with

 doi:10.20944/preprints202503.0750.v1

head and neck squamous cell carcinoma. *Cancer immunology, immunotherapy : CII* **2024**, *73*, 3, doi:10.1007/s00262-023-03595-0.

55. Zhang, W.; Liu, Y.; Yan, Z.; Yang, H.; Sun, W.; Yao, Y.; Chen, Y.; Jiang, R. IL-6 promotes PD-L1 expression in monocytes and macrophages by decreasing protein tyrosine phosphatase receptor type O expression in human hepatocellular carcinoma. *Journal for immunotherapy of cancer* **2020**, *8*, doi:10.1136/jitc-2019-000285.
56. Zhang, N.; Zeng, Y.; Du, W.; Zhu, J.; Shen, D.; Liu, Z.; Huang, J.A. The EGFR pathway is involved in the regulation of PD-L1 expression via the IL-6/JAK/STAT3 signaling pathway in EGFR-mutated non-small cell lung cancer. *International journal of oncology* **2016**, *49*, 1360-1368, doi:10.3892/ijo.2016.3632.
57. Hartley, G.; Regan, D.; Guth, A.; Dow, S. Regulation of PD-L1 expression on murine tumor-associated monocytes and macrophages by locally produced TNF-alpha. *Cancer immunology, immunotherapy : CII* **2017**, *66*, 523-535, doi:10.1007/s00262-017-1955-5.
58. Xiao, W.; Klement, J.D.; Lu, C.; Ibrahim, M.L.; Liu, K. IFNAR1 Controls Autocrine Type I IFN Regulation of PD-L1 Expression in Myeloid-Derived Suppressor Cells. *Journal of immunology* **2018**, *201*, 264-277, doi:10.4049/jimmunol.1800129.
59. Hirayama, A.; Tanaka, K.; Tsutsumi, H.; Nakanishi, T.; Yamashita, S.; Mizusaki, S.; Ishii, Y.; Ota, K.; Yoneshima, Y.; Iwama, E., et al. Regulation of PD-L1 expression in non-small cell lung cancer by interleukin-1beta. *Frontiers in immunology* **2023**, *14*, 1192861, doi:10.3389/fimmu.2023.1192861.
60. Zhong, F.; Cheng, X.; Sun, S.; Zhou, J. Transcriptional activation of PD-L1 by Sox2 contributes to the proliferation of hepatocellular carcinoma cells. *Oncology reports* **2017**, *37*, 3061-3067, doi:10.3892/or.2017.5523.
61. Parsa, A.T.; Waldron, J.S.; Panner, A.; Crane, C.A.; Parney, I.F.; Barry, J.J.; Cachola, K.E.; Murray, J.C.; Tihan, T.; Jensen, M.C., et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. *Nature medicine* **2007**, *13*, 84-88, doi:10.1038/nm1517.
62. Liu, J.; Hamrouni, A.; Wolowiec, D.; Coiteux, V.; Kuliczkowski, K.; Hetuin, D.; Saudemont, A.; Quesnel, B. Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-{gamma} and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. *Blood* **2007**, *110*, 296-304, doi:10.1182/blood-2006-10-051482.
63. Lastwika, K.J.; Wilson, W., 3rd; Li, Q.K.; Norris, J.; Xu, H.; Ghazarian, S.R.; Kitagawa, H.; Kawabata, S.; Taube, J.M.; Yao, S., et al. Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. *Cancer research* **2016**, *76*, 227-238, doi:10.1158/0008-5472.CAN-14-3362.
64. Minchom, A.; Thavasu, P.; Ahmad, Z.; Stewart, A.; Georgiou, A.; O'Brien, M.E.R.; Popat, S.; Bhosle, J.; Yap, T.A.; de Bono, J., et al. A study of PD-L1 expression in KRAS mutant non-small cell lung cancer cell lines exposed to relevant targeted treatments. *PloS one* **2017**, *12*, e0186106, doi:10.1371/journal.pone.0186106.
65. Ota, K.; Azuma, K.; Kawahara, A.; Hattori, S.; Iwama, E.; Tanizaki, J.; Harada, T.; Matsumoto, K.; Takayama, K.; Takamori, S., et al. Induction of PD-L1 Expression by the EML4-ALK Oncoprotein and Downstream Signaling Pathways in Non-Small Cell Lung Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2015**, *21*, 4014-4021, doi:10.1158/1078-0432.CCR-15-0016.
66. Noh, H.; Hu, J.; Wang, X.; Xia, X.; Satelli, A.; Li, S. Immune checkpoint regulator PD-L1 expression on tumor cells by contacting CD11b positive bone marrow derived stromal cells. *Cell communication and signaling : CCS* **2015**, *13*, 14, doi:10.1186/s12964-015-0093-y.
67. Coelho, M.A.; de Carne Trecesson, S.; Rana, S.; Zecchin, D.; Moore, C.; Molina-Arcas, M.; East, P.; Spencer-Dene, B.; Nye, E.; Barnouin, K., et al. Oncogenic RAS Signaling Promotes Tumor Immunoresistance by Stabilizing PD-L1 mRNA. *Immunity* **2017**, *47*, 1083-1099 e1086, doi:10.1016/j.immuni.2017.11.016.
68. Saigi, M.; Alburquerque-Bejar, J.J.; Mc Leer-Florin, A.; Pereira, C.; Pros, E.; Romero, O.A.; Baixeras, N.; Esteve-Codina, A.; Nadal, E.; Brambilla, E., et al. MET-Oncogenic and JAK2-Inactivating Alterations Are Independent Factors That Affect Regulation of PD-L1 Expression in Lung Cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2018**, *24*, 4579-4587, doi:10.1158/1078-0432.CCR-18-0267.

 doi:10.20944/preprints202503.0750.v1

69. Mezzadra, R.; Sun, C.; Jae, L.T.; Gomez-Eerland, R.; de Vries, E.; Wu, W.; Logtenberg, M.E.W.; Slagter, M.; Rozeman, E.A.; Hofland, I., et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. *Nature* **2017**, *549*, 106-110, doi:10.1038/nature23669.
70. Burr, M.L.; Sparbier, C.E.; Chan, Y.C.; Williamson, J.C.; Woods, K.; Beavis, P.A.; Lam, E.Y.N.; Henderson, M.A.; Bell, C.C.; Stolzenburg, S., et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. *Nature* **2017**, *549*, 101-105, doi:10.1038/nature23643.
71. Lim, S.O.; Li, C.W.; Xia, W.; Cha, J.H.; Chan, L.C.; Wu, Y.; Chang, S.S.; Lin, W.C.; Hsu, J.M.; Hsu, Y.H., et al. Deubiquitination and Stabilization of PD-L1 by CSN5. *Cancer cell* **2016**, *30*, 925-939, doi:10.1016/j.ccell.2016.10.010.
72. Lu, C.; Liu, K. Epigenetic regulation of PD-L1 expression and pancreatic cancer response to checkpoint immunotherapy. *Translational cancer research* **2017**, *6*, S652-S654, doi:10.21037/tcr.2017.05.32.
73. Hou, B.; Chen, T.; Zhang, H.; Li, J.; Wang, P.; Shang, G. The E3 ubiquitin ligases regulate PD-1/PD-L1 protein levels in tumor microenvironment to improve immunotherapy. *Frontiers in immunology* **2023**, *14*, 1123244, doi:10.3389/fimmu.2023.1123244.
74. Gou, Q.; Dong, C.; Xu, H.; Khan, B.; Jin, J.; Liu, Q.; Shi, J.; Hou, Y. PD-L1 degradation pathway and immunotherapy for cancer. *Cell death & disease* **2020**, *11*, 955, doi:10.1038/s41419-020-03140-2.
75. Wang, Y.; Sun, Q.; Mu, N.; Sun, X.; Wang, Y.; Fan, S.; Su, L.; Liu, X. The deubiquitinase USP22 regulates PD-L1 degradation in human cancer cells. *Cell communication and signaling : CCS* **2020**, *18*, 112, doi:10.1186/s12964-020-00612-y.
76. De, S.; Holvey-Bates, E.G.; Mahen, K.; Willard, B.; Stark, G.R. The ubiquitin E3 ligase FBXO22 degrades PD-L1 and sensitizes cancer cells to DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* **2021**, *118*, doi:10.1073/pnas.2112674118.
77. Yu, X.; Li, W.; Liu, H.; Wang, X.; Coarfa, C.; Cheng, C.; Yu, X.; Zeng, Z.; Cao, Y.; Young, K.H., et al. PD-L1 translocation to the plasma membrane enables tumor immune evasion through MIB2 ubiquitination. *The Journal of clinical investigation* **2023**, *133*, doi:10.1172/JCI160456.
78. Wei, M.; Mo, Y.; Liu, J.; Zhai, J.; Li, H.; Xu, Y.; Peng, Y.; Tang, Z.; Wei, T.; Yang, X., et al. Ubiquitin ligase RNF125 targets PD-L1 for ubiquitination and degradation. *Frontiers in oncology* **2022**, *12*, 835603, doi:10.3389/fonc.2022.835603.
79. Chemnitz, J.M.; Parry, R.V.; Nichols, K.E.; June, C.H.; Riley, J.L. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. *Journal of immunology* **2004**, *173*, 945-954.
80. Parry, R.V.; Chemnitz, J.M.; Frauwirth, K.A.; Lanfranco, A.R.; Braunstein, I.; Kobayashi, S.V.; Linsley, P.S.; Thompson, C.B.; Riley, J.L. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol* **2005**, *25*, 9543-9553.
81. Sheppard, K.A.; Fitz, L.J.; Lee, J.M.; Benander, C.; George, J.A.; Wooters, J.; Qiu, Y.; Jussif, J.M.; Carter, L.L.; Wood, C.R., et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. *FEBS Lett* **2004**, *574*, 37-41.
82. Patsoukis, N.; Bardhan, K.; Chatterjee, P.; Sari, D.; Liu, B.; Bell, L.N.; Karoly, E.D.; Freeman, G.J.; Petkova, V.; Seth, P., et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. *Nature communications* **2015**, *6*, 6692, doi:10.1038/ncomms7692.
83. Patsoukis, N.; Brown, J.; Petkova, V.; Liu, F.; Li, L.; Boussiotis, V.A. Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. *Science signaling* **2012**, *5*, ra46.
84. Patsoukis, N.; Li, L.; Sari, D.; Petkova, V.; Boussiotis, V.A. PD-1 increases PTEN phosphatase activity while decreasing PTEN protein stability by inhibiting casein kinase 2. *Mol Cell Biol* **2013**, *33*, 3091-3098.
85. Patsoukis, N.; Sari, D.; Boussiotis, V.A. PD-1 inhibits T cell proliferation by upregulating p27 and p15 and suppressing Cdc25A. *Cell Cycle* **2012**, *11*, 4305-4309.
86. Patsoukis, N.; Duke-Cohan, J.S.; Chaudhri, A.; Aksoylar, H.I.; Wang, Q.; Council, A.; Berg, A.; Freeman, G.J.; Boussiotis, V.A. Interaction of SHP-2 SH2 domains with PD-1 ITSM induces PD-1 dimerization and SHP-2 activation. *Communications biology* **2020**, *3*, 128, doi:10.1038/s42003-020-0845-0.

 doi:10.20944/preprints202503.0750.v1

87. Nurieva, R.; Thomas, S.; Nguyen, T.; Martin-Orozco, N.; Wang, Y.; Kaja, M.K.; Yu, X.Z.; Dong, C. T-cell tolerance or function is determined by combinatorial costimulatory signals. *Embo J* **2006**, *25*, 2623-2633.
88. Nurieva, R.I.; Zheng, S.; Jin, W.; Chung, Y.; Zhang, Y.; Martinez, G.J.; Reynolds, J.M.; Wang, S.L.; Lin, X.; Sun, S.C., et al. The E3 ubiquitin ligase GRAIL regulates T cell tolerance and regulatory T cell function by mediating T cell receptor-CD3 degradation. *Immunity* **2010**, *32*, 670-680, doi:10.1016/j.immuni.2010.05.002.
89. Chocarro, L.; Blanco, E.; Fernandez-Rubio, L.; Garnica, M.; Zuazo, M.; Garcia, M.J.; Bocanegra, A.; Echaide, M.; Johnston, C.; Edwards, C.J., et al. PD-1/LAG-3 co-signaling profiling uncovers CBL ubiquitin ligases as key immunotherapy targets. *EMBO molecular medicine* **2024**, *16*, 1791-1816, doi:10.1038/s44321-024-00098-y.
90. Azuma, T.; Yao, S.; Zhu, G.; Flies, A.S.; Flies, S.J.; Chen, L. B7-H1 is a ubiquitous antiapoptotic receptor on cancer cells. *Blood* **2008**, *111*, 3635-3643, doi:10.1182/blood-2007-11-123141.
91. Chang, C.H.; Qiu, J.; O'Sullivan, D.; Buck, M.D.; Noguchi, T.; Curtis, J.D.; Chen, Q.; Gindin, M.; Gubin, M.M.; van der Windt, G.J., et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* **2015**, *162*, 1229-1241, doi:10.1016/j.cell.2015.08.016.
92. Palmer, C.S.; Ostrowski, M.; Balderson, B.; Christian, N.; Crowe, S.M. Glucose metabolism regulates T cell activation, differentiation, and functions. *Frontiers in immunology* **2015**, *6*, 1, doi:10.3389/fimmu.2015.00001.
93. Kim, S.; Jang, J.Y.; Koh, J.; Kwon, D.; Kim, Y.A.; Paeng, J.C.; Ock, C.Y.; Keam, B.; Kim, M.; Kim, T.M., et al. Programmed cell death ligand-1-mediated enhancement of hexokinase 2 expression is inversely related to T-cell effector gene expression in non-small-cell lung cancer. *Journal of experimental & clinical cancer research : CR* **2019**, *38*, 462, doi:10.1186/s13046-019-1407-5.
94. Clark, C.A.; Gupta, H.B.; Sareddy, G.; Pandeswara, S.; Lao, S.; Yuan, B.; Drerup, J.M.; Padron, A.; Conejo-Garcia, J.; Murthy, K., et al. Tumor-Intrinsic PD-L1 Signals Regulate Cell Growth, Pathogenesis, and Autophagy in Ovarian Cancer and Melanoma. *Cancer research* **2016**, *76*, 6964-6974, doi:10.1158/0008-5472.CAN-16-0258.
95. Fierro, J., Jr.; DiPasquale, J.; Perez, J.; Chin, B.; Chokpapone, Y.; Tran, A.M.; Holden, A.; Factoriza, C.; Sivagnanakumar, N.; Aguilar, R., et al. Dual-sgRNA CRISPR/Cas9 knockout of PD-L1 in human U87 glioblastoma tumor cells inhibits proliferation, invasion, and tumor-associated macrophage polarization. *Scientific reports* **2022**, *12*, 2417, doi:10.1038/s41598-022-06430-1.
96. Gato-Canas, M.; Zuazo, M.; Arasanz, H.; Ibanez-Vea, M.; Lorenzo, L.; Fernandez-Hinojal, G.; Vera, R.; Smerdou, C.; Martisova, E.; Arozarena, I., et al. PDL1 Signals through Conserved Sequence Motifs to Overcome Interferon-Mediated Cytotoxicity. *Cell reports* **2017**, *20*, 1818-1829, doi:10.1016/j.celrep.2017.07.075.
97. Gupta, H.B.; Clark, C.A.; Yuan, B.; Sareddy, G.; Pandeswara, S.; Padron, A.S.; Hurez, V.; Conejo-Garcia, J.; Vadlamudi, R.; Li, R., et al. Tumor cell-intrinsic PD-L1 promotes tumor-initiating cell generation and functions in melanoma and ovarian cancer. *Signal transduction and targeted therapy* **2016**, *1*, pii: 16030.
98. Wang, F.; Yang, L.; Xiao, M.; Zhang, Z.; Shen, J.; Anuchapreeda, S.; Tima, S.; Chiampanichayakul, S.; Xiao, Z. PD-L1 regulates cell proliferation and apoptosis in acute myeloid leukemia by activating PI3K-AKT signaling pathway. *Scientific reports* **2022**, *12*, 11444, doi:10.1038/s41598-022-15020-0.
99. Deng, L.; Qian, G.; Zhang, S.; Zheng, H.; Fan, S.; Lesinski, G.B.; Owonikoko, T.K.; Ramalingam, S.S.; Sun, S.Y. Inhibition of mTOR complex 1/p70 S6 kinase signaling elevates PD-L1 levels in human cancer cells through enhancing protein stabilization accompanied with enhanced beta-TrCP degradation. *Oncogene* **2019**, *38*, 6270-6282, doi:10.1038/s41388-019-0877-4.
100. Chen, S.; Han, J.; Deng, H.; Lu, Y.; Wang, Z.; Zhang, Q.; Xia, R. Platelet PD-L1 inhibits storage-induced apoptosis by sustaining activation of the AKT signalling pathway. *Thrombosis research* **2024**, *240*, 109056, doi:10.1016/j.thromres.2024.109056.
101. Cao, Y.; Liang, W.; Fang, L.; Liu, M.K.; Zuo, J.; Peng, Y.L.; Shan, J.J.; Sun, R.X.; Zhao, J.; Wang, J. PD-L1/PD-L1 signalling promotes colorectal cancer cell migration ability through RAS/MEK/ERK. *Clinical and experimental pharmacology & physiology* **2022**, *49*, 1281-1293, doi:10.1111/1440-1681.13717.
102. Shin, D.S.; Zaretsky, J.M.; Escuin-Ordinas, H.; Garcia-Diaz, A.; Hu-Lieskovan, S.; Kalbasi, A.; Grasso, C.S.; Hugo, W.; Sandoval, S.; Torrejon, D.Y., et al. Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. *Cancer discovery* **2017**, *7*, 188-201, doi:10.1158/2159-8290.CD-16-1223.

 doi:10.20944/preprints202503.0750.v1

103. Garcia-Diaz, A.; Shin, D.S.; Moreno, B.H.; Saco, J.; Escuin-Ordinas, H.; Rodriguez, G.A.; Zaretsky, J.M.; Sun, L.; Hugo, W.; Wang, X., et al. Interferon Receptor Signaling Pathways Regulating PD-L1 and PD-L2 Expression. *Cell reports* **2017**, *19*, 1189-1201, doi:10.1016/j.celrep.2017.04.031.
104. Schiantarelli, J.; Benamar, M.; Park, J.; Sax, H.E.; Oliveira, G.; Bosma-Moody, A.; Campbell, K.M.; Liu, D.; Johnson, D.B.; Rodig, S., et al. Genomic mediators of acquired resistance to immunotherapy in metastatic melanoma. *Cancer cell* **2025**, *43*, 308-316 e306, doi:10.1016/j.ccell.2025.01.009.
105. Gao, Y.; Nihira, N.T.; Bu, X.; Chu, C.; Zhang, J.; Kolodziejczyk, A.; Fan, Y.; Chan, N.T.; Ma, L.; Liu, J., et al. Acetylation-dependent regulation of PD-L1 nuclear translocation dictates the efficacy of anti-PD-1 immunotherapy. *Nature cell biology* **2020**, *22*, 1064-1075, doi:10.1038/s41556-020-0562-4.
106. Yu, J.; Zhuang, A.; Gu, X.; Hua, Y.; Yang, L.; Ge, S.; Ruan, J.; Chai, P.; Jia, R.; Fan, X. Nuclear PD-L1 promotes EGR1-mediated angiogenesis and accelerates tumorigenesis. *Cell discovery* **2023**, *9*, 33, doi:10.1038/s41421-023-00521-7.
107. Wang, H.; Yao, H.; Li, C.; Shi, H.; Lan, J.; Li, Z.; Zhang, Y.; Liang, L.; Fang, J.Y.; Xu, J. HIP1R targets PD-L1 to lysosomal degradation to alter T cell-mediated cytotoxicity. *Nature chemical biology* **2019**, *15*, 42-50, doi:10.1038/s41589-018-0161-x.
108. Yang, Y.; Hsu, J.M.; Sun, L.; Chan, L.C.; Li, C.W.; Hsu, J.L.; Wei, Y.; Xia, W.; Hou, J.; Qiu, Y., et al. Palmitoylation stabilizes PD-L1 to promote breast tumor growth. *Cell research* **2019**, *29*, 83-86, doi:10.1038/s41422-018-0124-5.
109. Yao, H.; Lan, J.; Li, C.; Shi, H.; Brosseau, J.P.; Wang, H.; Lu, H.; Fang, C.; Zhang, Y.; Liang, L., et al. Inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. *Nature biomedical engineering* **2019**, *3*, 306-317, doi:10.1038/s41551-019-0375-6.
110. Grasso, C.S.; Tsoi, J.; Onyshchenko, M.; Abril-Rodriguez, G.; Ross-Macdonald, P.; Wind-Rotolo, M.; Champhekar, A.; Medina, E.; Torrejon, D.Y.; Shin, D.S., et al. Conserved Interferon-gamma Signaling Drives Clinical Response to Immune Checkpoint Blockade Therapy in Melanoma. *Cancer cell* **2021**, *39*, 122, doi:10.1016/j.ccell.2020.11.015.
111. Kalbasi, A.; Tariveranmoshabad, M.; Hakimi, K.; Kremer, S.; Campbell, K.M.; Funes, J.M.; Vega-Crespo, A.; Parisi, G.; Champekar, A.; Nguyen, C., et al. Uncoupling interferon signaling and antigen presentation to overcome immunotherapy resistance due to JAK1 loss in melanoma. *Science translational medicine* **2020**, *12*, doi:10.1126/scitranslmed.abb0152.
112. Traini, G.; Ruiz-de-Angulo, A.; Blanco-Canosa, J.B.; Zamacola Bascaran, K.; Molinaro, A.; Silipo, A.; Escors, D.; Mareque-Rivas, J.C. Cancer Immunotherapy of TLR4 Agonist-Antigen Constructs Enhanced with Pathogen-Mimicking Magnetite Nanoparticles and Checkpoint Blockade of PD-L1. *Small* **2019**, *15*, e1803993, doi:10.1002/smll.201803993.
113. Cheon, H.; Holvey-Bates, E.G.; McGrail, D.J.; Stark, G.R. PD-L1 sustains chronic, cancer cell-intrinsic responses to type I interferon, enhancing resistance to DNA damage. *Proceedings of the National Academy of Sciences of the United States of America* **2021**, *118*, doi:10.1073/pnas.2112258118.
114. Qu, P.; Li, X.; Liu, W.; Zhou, F.; Xu, X.; Tang, J.; Sun, M.; Li, J.; Li, H.; Han, Y., et al. Absence of PD-L1 signaling hinders macrophage defense against Mycobacterium tuberculosis via upregulating STAT3/IL-6 pathway. *Microbes and infection* **2024**, *26*, 105352, doi:10.1016/j.micinf.2024.105352.
115. Editorial: Gene cloning: one milestone on a very long road. *Lancet* **1976**, *1*, 893.
116. Hou, J.; Zhao, R.; Xia, W.; Chang, C.W.; You, Y.; Hsu, J.M.; Nie, L.; Chen, Y.; Wang, Y.C.; Liu, C., et al. PD-L1-mediated gasdermin C expression switches apoptosis to pyroptosis in cancer cells and facilitates tumour necrosis. *Nature cell biology* **2020**, *22*, 1264-1275, doi:10.1038/s41556-020-0575-z.
117. Cavazzoni, A.; Digiacomo, G.; Volta, F.; Alfieri, R.; Giovannetti, E.; Gnetti, L.; Bellini, L.; Galetti, M.; Fumarola, C.; Xu, G., et al. PD-L1 overexpression induces STAT signaling and promotes the secretion of pro-angiogenic cytokines in non-small cell lung cancer (NSCLC). *Lung cancer* **2024**, *187*, 107438, doi:10.1016/j.lungcan.2023.107438.
118. Passariello, M.; D'Alise, A.M.; Esposito, A.; Vetrei, C.; Froechlich, G.; Scarselli, E.; Nicosia, A.; De Lorenzo, C. Novel Human Anti-PD-L1 mAbs Inhibit Immune-Independent Tumor Cell Growth and PD-L1 Associated Intracellular Signalling. *Scientific reports* **2019**, *9*, 13125, doi:10.1038/s41598-019-49485-3.

 doi:10.20944/preprints202503.0750.v1

119. Ghosh, S.; Nataraj, N.B.; Noronha, A.; Patkar, S.; Sekar, A.; Mukherjee, S.; Winograd-Katz, S.; Kramarski, L.; Verma, A.; Lindzen, M., et al. PD-L1 recruits phospholipase C and enhances tumorigenicity of lung tumors harboring mutant forms of EGFR. *Cell reports* **2021**, *35*, 109181, doi:10.1016/j.celrep.2021.109181.
120. Jalali, S.; Price-Troska, T.; Bothun, C.; Villasboas, J.; Kim, H.J.; Yang, Z.Z.; Novak, A.J.; Dong, H.; Ansell, S.M. Reverse signaling via PD-L1 supports malignant cell growth and survival in classical Hodgkin lymphoma. *Blood Cancer J* **2019**, *9*, 22, doi:10.1038/s41408-019-0185-9.
121. Huttlin, E.L.; Ting, L.; Bruckner, R.J.; Gebreab, F.; Gygi, M.P.; Szpyt, J.; Tam, S.; Zarraga, G.; Colby, G.; Baltier, K., et al. The BioPlex Network: A Systematic Exploration of the Human Interactome. *Cell* **2015**, *162*, 425-440, doi:10.1016/j.cell.2015.06.043.
122. Sato, H.; Niimi, A.; Yasuhara, T.; Permata, T.B.M.; Hagiwara, Y.; Isono, M.; Nuryadi, E.; Sekine, R.; Oike, T.; Kakoti, S., et al. DNA double-strand break repair pathway regulates PD-L1 expression in cancer cells. *Nature communications* **2017**, *8*, 1751, doi:10.1038/s41467-017-01883-9.
123. Ozawa, N.; Yokobori, T.; Osone, K.; Katayama, C.; Suga, K.; Komine, C.; Shibasaki, Y.; Shiraishi, T.; Okada, T.; Kato, R., et al. PD-L1 upregulation is associated with activation of the DNA double-strand break repair pathway in patients with colitic cancer. *Scientific reports* **2021**, *11*, 13077, doi:10.1038/s41598-021-92530-3.
124. Shu, Z.; Dwivedi, B.; Switchenko, J.M.; Yu, D.S.; Deng, X. PD-L1 deglycosylation promotes its nuclear translocation and accelerates DNA double-strand-break repair in cancer. *Nature communications* **2024**, *15*, 6830, doi:10.1038/s41467-024-51242-8.
125. Polioudaki, H.; Chantziou, A.; Kalyvianaki, K.; Malamos, P.; Notas, G.; Mavroudis, D.; Kampa, M.; Castanas, E.; Theodoropoulos, P.A. Nuclear localization of PD-L1: artifact or reality? *Cellular oncology* **2019**, *42*, 237-242, doi:10.1007/s13402-018-00419-7.
126. Schulz, D.; Feulner, L.; Santos Rubenich, D.; Heimer, S.; Rohrmuller, S.; Reinders, Y.; Falchetti, M.; Wetzel, M.; Braganhol, E.; Lummertz da Rocha, E., et al. Subcellular localization of PD-L1 and cell-cycle-dependent expression of nuclear PD-L1 variants: implications for head and neck cancer cell functions and therapeutic efficacy. *Molecular oncology* **2024**, *18*, 431-452, doi:10.1002/1878-0261.13567.
127. Du, W.; Zhu, J.; Zeng, Y.; Liu, T.; Zhang, Y.; Cai, T.; Fu, Y.; Zhang, W.; Zhang, R.; Liu, Z., et al. KPNB1-mediated nuclear translocation of PD-L1 promotes non-small cell lung cancer cell proliferation via the Gas6/MerTK signaling pathway. *Cell death and differentiation* **2021**, *28*, 1284-1300, doi:10.1038/s41418-020-00651-5.
128. Kornepati, A.V.R.; Boyd, J.T.; Murray, C.E.; Saifetiarova, J.; de la Pena Avalos, B.; Rogers, C.M.; Bai, H.; Padron, A.S.; Liao, Y.; Ontiveros, C., et al. Tumor Intrinsic PD-L1 Promotes DNA Repair in Distinct Cancers and Suppresses PARP Inhibitor-Induced Synthetic Lethality. *Cancer research* **2022**, *82*, 2156-2170, doi:10.1158/0008-5472.CAN-21-2076.
129. Murray, C.E.; Kornepati, A.V.R.; Ontiveros, C.; Liao, Y.; de la Pena Avalos, B.; Rogers, C.M.; Liu, Z.; Deng, Y.; Bai, H.; Kari, S., et al. Tumour-intrinsic PDL1 signals regulate the Chk2 DNA damage response in cancer cells and mediate resistance to Chk1 inhibitors. *Mol Cancer* **2024**, *23*, 242, doi:10.1186/s12943-024-02147-z.
130. Nihira, N.T.; Wu, W.; Hosoi, M.; Togashi, Y.; Sunada, S.; Miyoshi, Y.; Miki, Y.; Ohta, T. Nuclear PD-L1 triggers tumour-associated inflammation upon DNA damage. *EMBO reports* **2025**, *26*, 635-655, doi:10.1038/s44319-024-00354-9.
131. Wang, X.; Wu, W.K.K.; Gao, J.; Li, Z.; Dong, B.; Lin, X.; Li, Y.; Li, Y.; Gong, J.; Qi, C., et al. Autophagy inhibition enhances PD-L1 expression in gastric cancer. *Journal of experimental & clinical cancer research : CR* **2019**, *38*, 140, doi:10.1186/s13046-019-1148-5.
132. Yu, Z.L.; Liu, J.Y.; Chen, G. Small extracellular vesicle PD-L1 in cancer: the knowns and unknowns. *NPJ precision oncology* **2022**, *6*, 42, doi:10.1038/s41698-022-00287-3.
133. Karwacz, K.; Arce, F.; Bricogne, C.; Kochan, G.; Escors, D. PD-L1 co-stimulation, ligand-induced TCR down-modulation and anti-tumor immunotherapy. *Oncoimmunology* **2012**, *1*, 86-88, doi:10.4161/onci.1.1.17824.
134. Pacheco-Torres, J.; Penet, M.F.; Mironchik, Y.; Krishnamachary, B.; Bhujwalla, Z.M. The PD-L1 metabolic interactome intersects with choline metabolism and inflammation. *Cancer & metabolism* **2021**, *9*, 10, doi:10.1186/s40170-021-00245-w.

135. Nieto, C.; Miller, B.; Alzofon, N.; Chimed, T.; Himes, J.; Joshi, M.; Gomez, K.; Chowdhury, F.N.; Le, P.N.; Weaver, A., et al. The programmed death ligand 1 interactome demonstrates bidirectional signaling coordinating immune suppression and cancer progression in head and neck squamous cell carcinoma. *Journal of the National Cancer Institute* **2023**, *115*, 1392-1403, doi:10.1093/jnci/djad126.
136. Schulz, D.; Stancev, I.; Sorrentino, A.; Menevse, A.N.; Beckhove, P.; Brockhoff, G.; Hautmann, M.G.; Reichert, T.E.; Bauer, R.J.; Ettl, T. Increased PD-L1 expression in radioresistant HNSCC cell lines after irradiation affects cell proliferation due to inactivation of GSK-3beta. *Oncotarget* **2019**, *10*, 573-583, doi:10.18632/oncotarget.26542.
137. Chen, C.; Li, S.; Xue, J.; Qi, M.; Liu, X.; Huang, Y.; Hu, J.; Dong, H.; Ling, K. PD-L1 tumor-intrinsic signaling and its therapeutic implication in triple-negative breast cancer. *JCI insight* **2021**, *6*, doi:10.1172/jci.insight.131458.
138. Ren, W.; Xu, Z.; Chang, Y.; Ju, F.; Wu, H.; Liang, Z.; Zhao, M.; Wang, N.; Lin, Y.; Xu, C., et al. Pharmaceutical targeting of OTUB2 sensitizes tumors to cytotoxic T cells via degradation of PD-L1. *Nature communications* **2024**, *15*, 9, doi:10.1038/s41467-023-44466-7.
139. Tu, X.; Qin, B.; Zhang, Y.; Zhang, C.; Kahila, M.; Nowsheen, S.; Yin, P.; Yuan, J.; Pei, H.; Li, H., et al. PD-L1 (B7-H1) Competes with the RNA Exosome to Regulate the DNA Damage Response and Can Be Targeted to Sensitize to Radiation or Chemotherapy. *Mol Cell* **2019**, *74*, 1215-1226 e1214, doi:10.1016/j.molcel.2019.04.005.
140. Theivanthiran, B.; Evans, K.S.; DeVito, N.C.; Plebanek, M.P.; Sturdivant, M.; Wachsmuth, L.P.; Salama, A.K.; Kang, Y.; Hsu, D.; Balko, J.M., et al. A tumor-intrinsic PD-L1-NLRP3 inflammasome signaling pathway drives resistance to anti-PD-1 immunotherapy. *The Journal of clinical investigation* **2020**, 10.1172/JCI133055, doi:10.1172/JCI133055.
141. Lucas, E.D.; Schafer, J.B.; Matsuda, J.; Kraus, M.; Burchill, M.A.; Tamburini, B.A.J. PD-L1 Reverse Signaling in Dermal Dendritic Cells Promotes Dendritic Cell Migration Required for Skin Immunity. *Cell reports* **2020**, *33*, 108258, doi:10.1016/j.celrep.2020.108258.
142. Schafer, J.B.; Lucas, E.D.; Dzieciatkowska, M.; Forward, T.; Tamburini, B.A.J. Programmed death ligand 1 intracellular interactions with STAT3 and focal adhesion protein Paxillin facilitate lymphatic endothelial cell remodeling. *The Journal of biological chemistry* **2022**, *298*, 102694, doi:10.1016/j.jbc.2022.102694.
143. Qiu, X.Y.; Hu, D.X.; Chen, W.Q.; Chen, R.Q.; Qian, S.R.; Li, C.Y.; Li, Y.J.; Xiong, X.X.; Liu, D.; Pan, F., et al. PD-L1 confers glioblastoma multiforme malignancy via Ras binding and Ras/Erk/EMT activation. *Biochimica et biophysica acta. Molecular basis of disease* **2018**, *1864*, 1754-1769, doi:10.1016/j.bbadis.2018.03.002.
144. Jeong, H.; Koh, J.; Kim, S.; Song, S.G.; Lee, S.H.; Jeon, Y.; Lee, C.H.; Keam, B.; Lee, S.H.; Chung, D.H., et al. Epithelial-mesenchymal transition induced by tumor cell-intrinsic PD-L1 signaling predicts a poor response to immune checkpoint inhibitors in PD-L1-high lung cancer. *British journal of cancer* **2024**, *131*, 23-36, doi:10.1038/s41416-024-02698-4.
145. Diskin, B.; Adam, S.; Cassini, M.F.; Sanchez, G.; Liria, M.; Aykut, B.; Buttar, C.; Li, E.; Sundberg, B.; Salas, R.D., et al. PD-L1 engagement on T cells promotes self-tolerance and suppression of neighboring macrophages and effector T cells in cancer. *Nature immunology* **2020**, *21*, 442-454, doi:10.1038/s41590-020-0620-x.
146. Fanelli, G.; Romano, M.; Nova-Lamperti, E.; Werner Sunderland, M.; Nerviani, A.; Scotta, C.; Bombardieri, M.; Quezada, S.A.; Sacks, S.H.; Noelle, R.J., et al. PD-L1 signaling on human memory CD4+ T cells induces a regulatory phenotype. *PLoS biology* **2021**, *19*, e3001199, doi:10.1371/journal.pbio.3001199.