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Programmed death ligand 1 signals in cancer cells

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Abstract

The paradigm of surface-expressed programmed death ligand 1 (PDL1) signalling to immune cell programmed death 1 (PD1) to inhibit antitumour immunity has helped to develop effective and revolutionary immunotherapies using antibodies blocking these cell-extrinsic interactions. The recent discovery of cancer cell-intrinsic PDL1 signals has broadened understanding of pathologic tumour PDL1 signal consequences that now includes control of tumour growth and survival pathways, stemness, immune effects, DNA damage responses and gene expression regulation. Many such effects are PD1-independent. These insights demonstrate that the prevailing cell-extrinsic PDL1 signalling paradigm is useful, but incomplete in important respects. This Perspective discusses historical and recent advances in understanding cancer cell-intrinsic PDL1 signals, mechanisms for signal controls and important immunopathologic consequences including resistance to cytotoxic agents, targeted small molecules and immunotherapies. Cancer cell-intrinsic PDL1 signals present novel drug discovery targets and also have potential as reliable treatment response biomarkers. Cancer cell-intrinsic PD1 signals and cell-intrinsic PDL1 signals in non-cancer cells are discussed briefly, as are PDL1 signals from soluble and vesicle-bound PDL1 and PDL1 isoforms. We conclude with suggestions for addressing the most pressing challenges and opportunities in this rapidly developing field.

Programmed death ligand 1 (PDL1; also known as CD274 and B7-H1) is an immune checkpoint molecule that was discovered to be expressed by heart, placenta, lung and skeletal muscle tissues and to regulate T cell proliferation and IL-10 secretion[1] in 1999. Soon after, researchers identified PDL1 expression on cancer cells and that PDL1 interaction with the receptor programmed death 1 (PD1) on T cells led to inhibition of T cell activation[2] including through induction of T cell apoptosis[3], thereby inhibiting antitumour immunity.

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Author contributions

A.V.R.K. and T.J.C. contributed equally to all aspects of the article. R.K.V. edited and fact-checked the manuscript.

Competing interests

T.J.C. and A.V.R.K. have filed a patent on using intracellular PDL1 to predict anticancer responses and drugs that reduce tumour PDL1 for treatment purposes.

Antibodies blocking PDL1 or PD1 improve antitumour immunity and prolonged survival in mouse cancer models and humans[3-9]. Immune checkpoint blockade (ICB) agents including these antibodies, as well as antibodies blocking the CTLA4 immune checkpoint, are now US Food and Drug Administration (FDA)-approved cancer immunotherapies. The research leading to their identification was recognized with a Nobel Prize in Medicine in 2018 shared by Jim Allison and Tasuku Honjo. The principal anticancer mechanism of anti-PDL1 or anti-PD1 antibodies is thought to be preventing the cell surface PDL1-mediated inhibition of $PD1^+$ antitumour T cells[3,10-12], thereby promoting antitumour immunity[13] (reviewed in REFS[6,14,15]). Although some patients with cancer experience durable and complete treatment responses from ICB, most fail to respond[6,16] and reliable anti-PDL1 or anti-PD1 treatment response biomarkers are lacking[8,9,14], suggesting the existence of alternative and/or additional PDL1-related immunopathogenesis and treatment resistance mechanisms.

The well-described cell-extrinsic immunopathogenic PDL1–PD1 pathway in cancer is represented by cancer or non-cancer cell surface-expressed PDL1 that extrinsically engages PD1 expressed on the surface of immune cells, leading to signalling downstream of PD1 to inhibit antitumour immunity. PDL1 reverse signalling refers to PD1 engaging cell surface PDL1 and induction of intracellular PDL1 signalling. Canonical PD1–PDL1 signals is defined here (and generally assumed in most literature) as PD1 signalling induced in immune cells by immune or tumour cell surface PDL1 to immune cell PD1 (REFS[6,14]) (BOX 1). Cancer cell-intrinsic PDL1 signals, that is, cellular functions induced by surface, cytosolic or nuclear PDL1, can be immunopathogenic, but are much less studied or understood, and have not previously been precisely defined.

Cell-intrinsic PDL1 signals can originate from PDL1 in specific subcellular locations (for example, cytosol and nucleus), often directed there by post-translational modifications (described later in the section Cancer cell-intrinsic signalling via intracellular PDL1), and might be elicited by surface PDL1–PD1 engagement, but can be PD1-independent. PDL1 signalling can originate from secreted, vesicular or exosomal PDL1 (REFS[17,18]) or non-stromal cells or platelets[19-22], and anti-surface PDL1 antibody can affect tumour-intrinsic PDL1 signals[23-28] through as yet incompletely defined mechanisms. This Perspective focuses on how cancer cell-intrinsic PDL1 signals control signalling biology within and among cancer cells (TABLE 1).

Cancer cell-intrinsic PDL1 effects were first demonstrated in 2008 as anti-apoptotic, through a PD1-dependent mechanism that required the PDL1 cytoplasmic tail, although the specific subcellular location was not defined[29]. This was followed by our 2016 demonstrations that cancer cell-intrinsic PDL1 exerts control of proliferation, immune-independent metastasis, mammalian target of rapamycin complex 1 (mTORC1) signalling, autophagy, small-molecule drug resistance and stemness in mouse and human ovarian cancer and melanoma cells[24,30]. Although specific subcellular PDL1 locations were not defined, we showed PD1 dependence for immune-independent, anti-PDL1-driven cancer cell proliferation suppression in vitro. Reports swiftly emerged that cancer cell-intrinsic PDL1 from unspecified subcellular locations in cancer cells promotes expression of distinct genes[24,31] and resistance to cytotoxic chemotherapy, radiation and other treatments[23,24,32], including anti-PD1 resistance[28], and suppresses signal transducer and activator of transcription

3 (STAT3) and interferon-β (IFNβ) signalling[27], specific immune cell recruitment[28], IFNγ resistance[33] and DNA damage[32]. Cytosolic PDL1 promotes expression of genes involved in the DNA damage response[34]. Nuclear PDL1 increases tumour pyroptosis[35], gene expression[35,36], anti-PD1 resistance[35], sister chromatid cohesion[37] and genomic stability[38]. None of these reports tested PD1 dependency in detail. Inhibiting cancer cell-intrinsic PDL1 signals with small molecules or antibodies for therapeutic benefit has been demonstrated[20,28,36,39].

Exploiting therapeutic vulnerabilities from cancer cell-intrinsic PDL1 signals or when soluble, vesicular, exosomal or aberrantly expressed, or its isoforms[17,40-45] (BOX 2, represents largely untapped opportunities warranting further investigation. Given the rapidly improving understanding of cell-intrinsic PDL1 signals, in this Perspective we discuss cancer cell-intrinsic PDL1 signals and suggest novel treatment strategies and response biomarkers. We cover mechanisms for cancer cell-intrinsic PDL1 signalling and major consequences. Finally, we address the targeting of tumour cell-intrinsic PDL1 signals in drug discovery.

PDL1 structure and immune cell PD1 engagement

PDL1 is a 290 amino acid, type 1 transmembrane protein in the immunoglobulin superfamily resembling the immunoglobulin light chain (FIG. 1a). It engages its major receptor PD1, in *trans* as well as in *cis*[46,47], to inhibit antitumour $PD1^+$ T cell functions[48], by antagonizing T cell receptor and CD28 co-stimulatory signals[49], authoritatively reviewed elsewhere[50]. PDL1 also engages its other known receptor, the immune co-signalling molecule CD80, in *trans*[51] as well as in *cis*[52] each to prevent T cell co-stimulation.

PDL1 domains.

PDL1 protein has five major domains (FIG. 1a). The domains lack canonical signalling motifs as observed in classical receptor tyrosine kinases but cell-intrinsic PDL1 signals can similarly be altered by post-translational modifications[35,36,53]. For example, PDL1 glycosylation enhances its protein stability[54] and binding to known interacting partners such as PD1 (REF.[50]), whereas PDL1 acetylation inhibits its nuclear translocation that suppresses anti-PD1 efficacy[36]. Cell-intrinsic PDL1 signals can come from full-length 290 amino acid PDL1 found at the cell surface as well as the cytosol and nucleus[35,36]. As surface-expressed PDL1 still contains a short 30 amino acid cytoplasmic tail[27,55], studies distinguishing cell-intrinsic signalling effects derived from surface versus fully cytosolic PDL1 must account for this mechanistic possibility if subcellular effects are considered. Mouse and human PDL1 share high protein sequence and structural similarity, although differences in sequence can affect anti-PDL1 binding and PD1 engagement[56]. Anti-PDL1 can increase CD80 signals to activate antitumour T cells[57] but specific consequences are little understood as the PDL1–CD80 interaction is much less studied compared with the PD1–PDL1 interaction.

Human PDL1 isoform 1 is the full-length protein generally referred to as ‘PDL1’, a convention used here. When fully intracellular, its Ig-V and Ig-C domains (which are extracellular when PDL1 is on the cell surface) are intracellular and can participate

directly in cell-intrinsic PDL1 signalling. At least five other PDL1 isoforms are described, all in human but not mouse cells, some having signal functions and/or prognostic significance[41-43,58] (FIG. 1a). A secreted carboxy-terminal tail-deficient isoform was detected in relapsed non-small-cell lung cancer that generated ICB resistance by competing for anti-PDL1 (REF.[59]). Additional functions of these PDL1 isoforms, including cell-intrinsic consequences and mechanisms, merit more investigation.

Most commercial anti-PDL1 antibodies recognize the Ig-V domain which engages PD1 (REF.[46]), and thereby block PD1 interaction, but do not recognize PDL1 isoforms lacking the Ig-V (PD1-binding) domain such as isoform 2 (which lacks the amino-terminal Ig-V moiety)[58]. Some human anti-PDL1 detection antibodies that recognize the C-terminal tail (for example, clones 405.9A11, E1L3N) are commercially available[60], do not interfere with PD1 engagement and should theoretically bind to PDL1 isoform 2, but that has not been reported. Similar limitations can apply to detecting other PDL1 isoforms.

Cancer cell-extrinsic PDL1-related treatment outcomes.

Cancer cell-extrinsic PDL1-related treatment outcomes have been reviewed extensively[14,61-63]. In brief, the principal anticancer mechanism of anti-PDL1 or anti-PD1 is thought to be preventing $PD1^+$ antitumour T cells from inhibition by cell surface-expressed PDL1 (REFS[3,10-12]), resulting in T cell reinvigoration[13], reduced T cell exhaustion or death and increased T cell memory and intratumoural antitumour immune cell infiltrates[6,14,15], including effects in chronic infection[64] that parallel cancer data.

Signalling mechanisms

Although PDL1 is generally considered to be surface-expressed, it is found in many subcellular compartments (FIG. 1b. Cytoplasmic PDL1 can be recycled from endosomes to the cell surface[65,66]. Functional nuclear PDL1 has been reported[35-37], but its cellular source remains unresolved. Although there are profound differences in cell-extrinsic versus cell-intrinsic PDL1 signals, the same PDL1 molecule could mediate either signal depending on subcellular location (BOX 1; FIG. 1b. The conformation of PDL1 on the cell surface (as well as in soluble form[45] or in exosomes[17,18]) is immunoglobulin-like and three-dimensional. This conformation is likely lost in fully intracellular PDL1 (although linear Ig-V and Ig-C domains could remain largely unchanged), which we predict affects interactions with potential intracellular binding partners. For example, PDL1–PD1 engagement is through their immunoglobulin-like regions[46] that require complex tertiary conformations which would be lost in intracellular compartments. Thus, known PD1-dependent cell-intrinsic PDL1 effects[24,29] likely can only occur through surface-expressed PDL1 (FIG. 1b. In addition, post-translational modifications directing PDL1 to distinct subcellular compartments or arising in such compartments can affect PDL1 signalling, such as acetylation, reducing nuclear PDL1 to improve anti-PD1 efficacy in a heterotopic colon cancer model[36] (FIG. 2. Cell-intrinsic PDL1 signals observed in cancer cells have also been detected in non-cancer cells and in the non-cancer, healthy context including natural killer cells[67], T cells[68], adipocytes[20], arterial smooth muscle cells[69] and dendritic cells[70], implicating PDL1 as a homeostatic molecule. We anticipate that PDL1 signals in

non-cancerous cells could differ from malignant counterparts and from other tumour genetic backgrounds and histologies just as PDL1 signal effects in cancer cells can differ among tumour types (BOX 1; TABLE 1. Much work in these contexts is merited to understand pathologic versus homeostatic outcomes. Additional PDL1 moiety signalling features are described in BOX 3.

Cancer cell-intrinsic signalling via surface PDL1.

Surface PDL1 signalling includes engagement of immune cell PD1 (REF.[2]) or CD80 (REF.[51]), but reports on functional CD80 engagement with PDL1 are limited. Although some cancer cells express PD1 (REFS[24,71,72]) or CD80 (REF.[73]), *cis* interactions with PDL1 have not yet been reported in cancer cells. In melanoma, PD1 on cancer cells can interact with cancer cell surface PDL1 leading to mTORC1 activation, cell proliferation and in vivo tumour growth[71] (FIG. 1b; TABLE 1). Whereas triple-negative breast cancer (TNBC) cells can also express both PD1 and PDL1, similar interactions leading to intrinsic PDL1 signalling have not been reported, although PDL1 contributed to proliferation and growth in vitro and in vivo[37] which could be, in part, because of cell type-specific differences in the levels of PD1 N-glycosylation that affect PDL1 engagement (as shown in T cells)[74] or other post-translational modifications of PDL1 that affect PD1 engagement. For example, a study of human MDA-MB-231 TNBC cells observed markedly reduced glycosylation of surface PDL1 relative to nuclear PDL1 (REF.[36]) implicating PDL1 glycosylation, or potentially other post-translational modifications, as a determinant of cell-intrinsic PDL1 signalling from the cell surface versus other subcellular compartments.

Therapeutically, cancer cell-intrinsic signalling in *trans* via the PD1-PDL1 axis on cancer cells may partially explain immune-independent anti-PDL1-mediated inhibition of proliferation in vitro in melanoma[24,71] and ovarian cancer[24] and in vivo in melanoma in NSG mice[24,71]. The transduction of cell-intrinsic signals by surface PDL1 occurs through cancer cell-expressed PD1 immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif sites, and can drive cell growth via phospho-S6 ribosomal protein (p-S6) in melanoma cells[71] (FIG. 1b), although the activity of this axis requires validation in other tumour types. By contrast, cancer cell-intrinsic PD1–PDL1 signalling, possibly in *trans*, in non-small-cell lung cancer cells in vitro and in vivo in NSG mice instead led to growth suppression by antagonizing p38 mitogen-activated protein kinase (MAPK)-extracellular-signal-regulated kinase (ERK) signals[72]. Although in vitro culture conditions appear similar in these distinct reports, more work is needed to clarify these discordant data which likely include effects from specific genetic backgrounds, tumour biology or cell-specific post-translational PDL1 or PD1 modifications[35,36,53,75].

Potential contributions of CD80 on cancer cells to cell-intrinsic PDL1 signals or functional outputs have not been reported. However, cancer cell surface PDL1 seems to induce cancer cell-intrinsic signals in some contexts through non-PD1 receptors such as β4 integrin on various human cervical cancer cells in vitro[76]. In various human bladder cancer cells, surface PDL1 interacted with surface β6 integrin to drive cell proliferation via FAK signalling and resistance to cisplatin-induced apoptosis in vitro[33]. PD1-induced cancer cell surface PDL1 reverse signalling, likely through the PDL1 cytoplasmic tail, has been

reported in tumours including Hodgkin lymphoma, driving cell growth and death resistance in this and other human lines in vitro[29,77]. Reverse signals likely also include as yet unreported cell-specific and/or context-specific differences. Anti-PDL1 antibody effects on tumour-intrinsic PDL1 signals[23-28,78] are discussed later in Interrupting cell-intrinsic PDL1 signals.

Cancer cell-intrinsic signalling via intracellular PDL1.

Direct protein–protein interactions are now a major known mechanism for cell-intrinsic PDL1 signal control in cancer cells. Interactions of PDL1 with glutathione synthase kinase 3β (GSK3β) and β-transducin repeats-containing protein (β-TRCP)[54], CKLF-like MARVEL transmembrane domain-containing protein 6 (CMTM6)[65,66] or CMTM4 (REF.[66]) can regulate total intracellular PDL1 content by reducing PDL1 protein degradation globally through proteasomal or lysosomal pathways. Other interactions, including with histone deacetylase 2 (HDAC2)[36] and Huntingtin-interacting protein 1-related protein (HIP1R)[79], can facilitate surface PDL1 relocation to intracellular compartments such as the cytosol, nucleus or lysosome through clathrin-mediated endocytosis[36]. When intracellular, other interactions with vimentin, p-STAT3 (Y703) and karyopherin B1 (KPNB1) can additionally promote, whereas p300 inhibits PDL1 nuclear translocation[35,36,80]. Once nuclear, PDL1 can promote transcription of pro-tumour genes such as *Gas6* (REF.[80]) (FIG. 1b; TABLE 1). Nuclear PDL1 can alter gene expression through specific DNA promoter region binding (mapped by PDL1 chromatin immunoprecipitation and deep sequencing)[36], possibly by acting as a co-factor to known transcription factors[35,36,80] such as specificity protein 1 (SP1).

Remarkably, cytosolic PDL1 has also been shown to bind to and stabilize mRNAs, thereby increasing expression of specific DNA damage repair proteins containing GAAGAA/U motifs in MDA-MB-231 cells[34]. Perinuclear or nuclear PDL1 associated with DNA-dependent protein kinase (DNA-PK) to activate MAPK or ERKs and promote cell survival signals in a cancer-specific manner also in MDA-MD-231 TNBC cells[81]. These latter two mechanisms appear to promote resistance to selected cytotoxic chemotherapies in vitro independent of PD1 or CD80. However, additional mechanistic studies are required to determine formally whether these competing cell-intrinsic PDL1 signalling consequences indeed mediate resistance to DNA damaging agents and to assess generalizability of such findings. As DNA damage can induce nuclear PDL1 accumulation[82] and PDL1 can also bind DNA directly[36], we predict that some control of DNA damage repair and, possibly chemoresistance occurs through direct participation of nuclear PDL1 in DNA repair pathways (for example, homologous recombination), although no mechanistic details have been reported.

Other protein interactions with intracellular PDL1 have been reported. However, the consequences on cancer cell functions of such interactions are incompletely understood and some studies are largely correlative. Such PDL1 interactions include with adaptin β2, keratins, importin α1, REL-associated protein (RelA; also known as p65) and interferon-regulated factors (IRFs) (all in REF.[36] with some additional interactions discovered using PDL1 co-immunoprecipitation mass spectrometry), protein kinase B (PKB; also known as

AKT)[83], HRAS[84], actin[36,83], protein-tyrosine phosphatase 1B (PTP1B)[85] and the Nijmegen breakage syndrome protein 1 (NBS1) involved in DNA repair[86]. Molecular, biochemical or functional studies, which should involve specific binding defective PDL1 mutants, are needed to understand the full phenotypic extent and formally prove the functional significance of these observed protein–protein interaction involving PDL1.

Intracellular PDL1 can also interact with large macromolecular structures, including with the nuclear cohesin complex to facilitate sister chromatid cohesion[37] and with cohesin subunit SA1 (SA1) to suppress genomic instability and/or aneuploidy[38]. The former study provided strong molecular evidence for intracellular PDL1 promotion of genomic stability by utilizing a PDS5 cohesin-associated factor B binding-defective PDL1 point mutant that failed to restore sister chromatid cohesion fully in *CD274*-knockout MDA-MB-231 cells. By contrast, PDL1 can associate with lysosomes[79], autophagosomes[24] and seven distinct oligomeric Golgi proteins[87] but intrinsic cellular and phenotypic effects of these interactions remain unclear. Potential effects include PDL1-mediated suppression (melanoma, ovarian cancer) or promotion (bladder cancer) of autophagy[23,24]. PDL1 association with autophagosomes suggests a potential mechanistic autophagy control effect requiring investigation. This exceptional diversity of potential interactions and signal outcomes makes a simple, unifying view of PDL1-regulated gene product expression difficult, especially as most outcomes do not appear to involve canonical signal motifs. Much work is needed to understand why and how PDL1 binds to and regulates so many diverse molecules and to understand its manifold roles in homeostatic versus dysfunctional processes. We speculate that PDL1 participation in phase separation[88] could help to provide a basis for this large array of macromolecular interaction effects. BOX 3 describes specific PDL1 domains mediating cancer cell-intrinsic signalling from intracellular PDL1.

Cell-intrinsic PDL1 from undefined subcellular locations.

Cancer cell-intrinsic PD1 engages PDL1 for mTORC1 activation in melanoma cells[71] and can also promote cancer cell proliferation[23,24]. Of note, we showed a role of cancer cell-intrinsic, PDL1-driven, immune-independent promotion of B16 melanoma metastasis[24], which could be from mTORC1-mediated cell proliferation or due to reported activation of epithelial to mesenchymal transition in TNBC, glioblastoma multiforme and carcinomas of the nasopharynx, lung and colon[31,43,84,85,89,90] that could affect invasion and motility. Cancer cell-intrinsic PDL1 control of pro-growth mTORC1 signalling was discussed above, demonstrating that PDL1-driven mTORC1 activation and autophagy suppression are not necessarily related and that PDL1 effects can differ by tumour type. PDL1-mediated mTOR control mechanisms have not been reported in detail, including relative effects on and consequences of downstream mTORC1 targets, areas for investigation.

In certain tumours, cancer stem cells have higher PDL1 expression levels than non-stem cells[30]. Cancer cell-intrinsic PDL1 promotes tumour stemness-like properties by increasing expression of canonical stemness genes (for example, *Oct4* and *Nanog*), self-renewal and in vivo tumorigenesis in mouse and human melanoma and ovarian cancer cells[30]. However, more specific mechanisms for PDL1-mediated control of stemness properties and

consequences for tumour pathogenesis or treatment resistance have not been reported in detail.

Cancer cell-intrinsic PDL1 immune effects

Immune outcomes from cancer cell surface PDL1 engaging immune cell surface PD1 are well described and are a basis for FDA-approved ICB antibodies[8,9]. By contrast it is little appreciated that cancer cell-intrinsic PDL1 signalling can also alter anticancer immunity. For example, as mentioned in the previous section, cell-intrinsic PDL1 can inhibit STAT3 activation (FIG. 1b) to inhibit sensitivity to IFNα, IFNβ and IFNγ and their signals in mouse melanoma cells in vitro[27], promote tumour immunogenic cell death through pyroptosis in vitro in human and in vivo in mouse TNBC[35], and suppress FAS–FASL-mediated apoptosis in a mouse mastocytoma model in vitro[29]. Interestingly, cancer cell-intrinsic nuclear PDL1 can promote transcription of major histocompatibility complex (MHC) class 1 expression in various human TNBC cell lines in vitro[36,91] that theoretically could inhibit natural killer cells or improve antigen-specific immune recognition (TABLE 1). Cancer cell-intrinsic PDL1 could also control antigen processing or presentation based on its known autophagic control[23,24] contributing to antigen processing[92].

Although nuclear PDL1 can upregulate MHC class 1 expression, which could enhance antitumour immune responses, it can simultaneously increase transcription of inhibitory immune checkpoint molecules (for example, V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA), B7-H3 and galectin-9) in various human TNBC cells in vitro[36] that could counteract the effects of the former and reduce ICB efficacy. These findings suggest opposing consequences of cell-intrinsic PDL1 on antitumour immune responses, the net effect of which could differ in distinct tumours, environments or treatments. In vivo, nuclear (de-acetylated) PDL1 was shown to mediate anti-PD1 resistance through as yet undefined mechanisms which result in reduced activated tumour-infiltrating T cells in heterotopic MC38 colon cancer[36] and, possibly, by increasing hypoxia-dependent, tumour-associated inflammation in 4T1 murine TNBC[35].

Cancer cell-intrinsic PDL1 signals can also regulate the DNA damage response in human breast and colon cancer cells[34] that could supress accumulation of mutations[14] and/or sensing of damaged nucleic acid in the cytoplasm (for example, by cyclic GMP–AMP synthase (cGAS)/stimulator of interferon genes (STING)), both potentially affecting tumour immunogenicity[93], or could promote anti-PD1 resistance through NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome-dependent recruitment of immunosuppressive cell subsets including macrophages as shown in a murine *BRAF*^{V600E}/*PTEN* melanoma model[28]. Cancer cell-intrinsic PDL1 controls tumour nuclear factor-κB (NF-κB) signalling[36], and we speculate downstream immune cell trafficking effects through differential chemokine expression as noted in RNA-sequencing data sets of PDL1 knockdown versus control MC38 tumours[91]. Thus, targeting cell-intrinsic PDL1 signals could further enhance the known immune potentiating of selected agents, for example poly(ADP-ribose) polymerase (PARP) inhibitors[94], a possibility that remains largely untested.

Therapeutic anti-PDL1 antibodies designed to disrupt cell surface PDL1/PD1 ligation can also alter some cancer cell-intrinsic PDL1 signals. We reported that anti-PDL1 directly suppresses melanoma, ovarian and bladder cancer cell proliferation in vitro, including potential tumour PD1 participation independent of immunity[23,24]. Anti-PDL1 antibodies could improve melanoma sensitivity to type I interferons independent of PD1 (REF.[27]), and activate STAT3-mediated suppression of pro-tumour NLRP3 inflammasome in mouse melanoma cells in vitro[27]. Because melanoma PDL1 promotes NLRP3 activation to augment anti-PD1 resistance in vivo[28], cell-intrinsic PDL1 targeting with anti-PDL1 antibodies to reverse this mechanism of resistance to anti-PD1 seems possible but has not been explored, which could include combining anti-PD1 with anti-PDL1. An RNA-sequencing study tested atezolizumab (human anti-PDL1) effects on gene expression in human MDA-MB-231 TNBC in vitro to show that it reduced epithelial–mesenchymal transition and cell-growth and hypoxia pathways[78], suggesting unappreciated cell-intrinsic effects of clinically utilized anti-PDL1 agents that require study.

An anti-PDL1 antibody increased tumour cell apoptosis and sensitivity to cytotoxic chemotherapies in vitro[81], but as this antibody was later shown to deplete total tumour cell PDL1 by suppressing its interaction from CMTM6 to increase lysosomal PDL1 degradation[34], the mechanism for apoptosis induced by anti-PDL1 here needs clarification. Specifically, the effect of anti-PDL1 blocking antibodies altering cell-intrinsic PDL1 signals could be due to disruption of non-canonical signals generated from surface PDL1 or may, consequently, result from altered subcellular redistribution of surface PDL1 to the cytoplasm or nucleus. Tumour PD1 or CD80 contributions and other mechanistic details for these novel anti-PDL1 effects are largely unreported.

Metabolic effects of cancer cell-intrinsic PDL1 can theoretically increase sarcoma capacity to outcompete T cells for glucose, likely by promoting mTORC1 activation in sarcoma cells, leading to reduction of T cell antitumour efficacy[95]. Some metabolic effects were induced by anti-PDL1 antibody How these cell-intrinsic PDL1 signals affect antitumour immunity or ICB efficacy in vivo requires further investigation.

Of note, some cancer cell-intrinsic PDL1 signals, especially from intracellular PDL1, are clearly refractory to inhibition by anti-PDL1 antibodies (for example, chromosome maintenance by sister chromatid cohesion)[37]. Interestingly, in contrast to a study in melanoma cells, anti-PDL1 (and anti-PD1) augmented human non-small-cell lung cancer proliferation in vitro, possibly by activating AKT–ERK1/2 signals[72], and could also explain some tumour type and context-specific anti-PDL1 treatment failures. Thus, new ways to target pathologic cell-intrinsic PDL1 signals in cancer cells which could drive immunotherapy resistance and rethinking of efficacy mechanisms for anti-PDL1 (and anti-PD1) ICB and the meaning of tumour PDL1 status as an ICB biomarker are needed.
As immune cells also express PDL1 that generates cell-intrinsic signals[67,68,70] (BOX 2), additional work investigating such effects on antitumour immunity and ICB response is warranted.

Cellular regulation of PDL1 content

Cell-autonomous regulation of cell-intrinsic and subcellular PDL1 content.

Factors and mechanisms controlling cancer cell or immune cell PDL1 expression have been comprehensively reviewed[53,96,97], and major endogenous PDL1 regulation controls are shown in FIG. 2. Although not well appreciated, total cancer cell PDL1 content is dependent on the cell cycle, with relatively increased PDL1 levels during the G2/M phase in human MDA-MB-231 TNBC cells[37] and decreased levels during the G1 phase in human MDA-MB-231 and HCC1954 TNBC and human HeLa cervical cancer lines[98], all in vitro. Nuclear PDL1 likely accumulates in the G2/M phase and is consistent with cell-intrinsic, nuclear PDL1 control of sister chromatid cohesion/chromosomal stability in MDA-MB-231 cells[37] but has yet to be assessed. Similarly, clinically observed PDL1 3′ untranslated region truncation leads to high cancer cell total PDL1 expression largely seen in certain leukaemias, lymphomas and gastric adenocarcinomas, associated with higher progression, but not affecting anti-PDL1 responsiveness[44]. Although subcellular-specific PDL1 expression has also not been explored in these studies, we predict that such augmented PDL1 expression could promote observed tumour progression by increasing cell-intrinsic PDL1 signals.

PDL1 distribution between the cell surface and cytosol is regulated homeostatically by CMTM4/CMTM6/TRAPPC4 (REFS[65,66,99]) and HIP1R[79] that promote or inhibit (respectively) endosomal to surface PDL1 recycling (FIG. 2). Thus, tumours that express high levels of endosomal recycling scaffolds such as CMTM6, as shown in MEL-53 human melanoma cells in vitro[65], can suppress antitumour immunity. Genetic silencing of *Trappc4* reduced PDL1 expression in mouse colon epithelial cells to reduce carcinogen-induced colon cancer, and in a heterotopic mouse MC38 colon cancer model to improve anti-PDL1 efficacy in vivo and to improve T cell cytotoxicity against human RKO colon cancer cells in vitro[99]. In both cases, silenced genes reduced tumour surface PDL1 by promoting its lysosomal degradation. By contrast, tumours with high HIP1R expression could have increased cytoplasmic or nuclear, relative to surface, PDL1. Although CMTM6, TRAPPC4 or HIP1R could regulate surface PDL1 content homeostatically, we note here that certain exogenous cellular stressors such as cytotoxic doxorubicin[82] or other chemotherapies[35] could also facilitate the redistribution of surface PDL1 to the cytosol or nucleus, originally observed in MDA-MB-231 TNBC[82], but the mechanistic underpinning of such effects remains to be elucidated.

It had been previously suggested that nuclear PDL1 was an artefact of immunofluorescence microscopy[100], but the existence of functional nuclear PDL1 has since been reported in convincing studies using nuclear localization-defective PDL1 mutants[35,36]. For example, post-translational HDAC2-dependent de-acetylation of the PDL1 C-terminal tail at K263 or the PDL1 K263 point mutant reduced nuclear PDL1 levels, whereas p300-dependent acetylation opposes this effect[36]. The observation that vimentin^{+} circulating cancer cells in patients with metastatic colon cancer showed nuclear PDL1 expression was of unclear functional importance but suggested that factors controlling epithelial–mesenchymal transition can facilitate nuclear PDL1 accumulation. In support, the same study describing acetylated PDL1 showed that incubation of human HCC1937 TNBC cells with transforming

growth factor-β (TGFβ) increased nuclear PDL1 expression[35], presumably by promoting vimentin expression. Vimentin contributes to promoting nuclear PDL1 translocation through a mechanism that may involve acetylation of surface-derived PDL1, but it likely facilitates general nuclear-cytoplasmic shuttling of PDL1 derived from other non-surface subcellular locations (for example, cytosol) independent of acetylation[36], which requires additional investigation.

Additional mechanisms of nuclear PDL1 entry have also been rigorously demonstrated. Hypoxia-induced binding of p-STAT3 to the human PDL1 C-terminal tail increases PDL1 nuclear localization, which may be augmented by many environmental and anticancer agents in breast cancer in vitro[35]. This study identified putative PDL1 nuclear localization and retention sequences[35]. Whether PDL1 nuclear entry always requires a binding partner warrants more experimentation. Although PDL1 C-terminal tail post-translational modifications augment PDL1 nuclear localization[35,36], factors mediating PDL1 nuclear import versus those regulating PDL1 nuclear retention or protein stability as seen in other nuclear protein regulation (for example, breast cancer type 1 susceptibility protein (BRCA1)[101] and androgen receptor[102]) have not been reported to date. PDL1 nuclear translocation in lung cancer was facilitated through KPNB1 binding to promote GAS6–tyrosine-protein kinase MER (MERTK signalling)[80] but whether this mechanism is dependent on acetylation or the PDL1 C-terminal tail is not known. We anticipate that moieties aside from the PDL1 C-terminal tail could increase PDL1 nuclear localization and mediate related signals, likely depending on specific tumour type and genetic backgrounds, which are areas warranting additional investigation.

The cellular source for nuclear PDL1 remains unresolved. Nuclear PDL1 could result from post-translationally modified PDL1 in the endoplasmic reticulum and/or Golgi followed by clathrin-mediated endocytosis from the surface, as with other surface proteins such as extracellular growth factor receptor (EGFR)[103], or directly from cytosolic PDL1 synthesized by free/unbound ribosomes (FIG. 2). One study reported hyper-N-glycosylated (~150 kDa) nuclear PDL1 compared with cytosolic or membrane-bound PDL1 (~55 kDa) in MDA-MB-231 human TNBC cells[37], suggesting that hyper-glycosylation might increase nuclear PDL1 translocation and/or retention and implicates a unique endoplasmic reticulum/Golgi-dependent, cell surface-independent pathway for nuclear PDL1 accumulation (FIG. 2). Discordantly, however, another report also using MDA-MB-231 cells did not observe a 150 kDa species of PDL1 in the nucleus and found that PDL1 glycosylation was dispensable for nuclear localization, rigorously demonstrated using a glycosylation-defective (4NQ) mutant PDL1 and subcellular fractionations[36], further underscoring the need for additional studies of PDL1 subcellular localization control. The specific mechanisms for nuclear PDL1 accumulation and retention appear context specific and can be further altered by certain cellular stress factors. The source or site of post-translational modifications affecting nuclear PDL1 content remains incompletely understood.

Potential therapeutic strategies

General considerations.

Although cytokines, notably IFNγ and tumour necrosis factor (TNF), are well known to augment PDL1 expression, studies generally do not distinguish effects on surface versus other subcellular locations. One study reported that IFNγ is less effective in inducing tumour nuclear PDL1 than TNF[35]. Additional work is needed to understand whether these cytokines can drive specific cell-intrinsic PDL1 signalling outcomes such as chemoresistance, including studying effects in distinct tumour types and immune cells. Indeed, in vivo tumour PDL1 induced by IFNγ (primarily derived from T cells) compared with induction by TNF (mainly derived from myeloid cells) elicited distinct tumour signals and tumour biological behaviour[104]. We speculate that differential outcomes could be, in part, the result of differential subcellular PDL1 distribution elicited by distinct cytokines. For example, TGFβ can increase nuclear PDL1 in some human breast cancer cell lines in vitro, which could be vimentin-dependent in TNBC[36], whereas IFNγ appears to promote cell-intrinsic surface PDL1-driven NLRP3 inflammasome activation[28]. In addition, we reported sexually dimorphic PDL1 effects on anti-PDL1 immunotherapy and cytokine production in B16 melanoma in vitro and in vivo[70], suggesting potential PDL1–sex hormone interactions which require investigation. IFNγ induces PDL1 largely through transcription whereas TNF induces PDL1 largely through protein stabilization by inhibiting its ubiquitination[105]. As cytokines such as TNF and IFNγ have pleiotropic effects, the ability to target cancer cell-intrinsic PDL1 signals elicited by specific cytokine microenvironmental factors will depend on better mechanistic understanding of their net effects and influence on PDL1 post-translational modifications.

Optimal targeting of cancer cell-intrinsic PDL1 signals requires a thorough understanding of these signals and their consequences, of signals in specific tumour types, in individual tumours from a given host owing to mutational and microenvironmental differences, and of off-target or secondary treatment effects. Considerations here are based on best available data at the time of writing that will surely change as new information becomes available. As it is unlikely that single treatment approaches will cure most cancers, combination strategies merit consideration based on understanding specific cell-intrinsic versus cell-extrinsic PDL1 signals in given settings.

Cancer cell-intrinsic PDL1 signals can be exploited clinically in three areas: as therapies alone or combined with other agents, as treatment response biomarkers or as prognosis biomarkers. No strategy specifically depleting tumour PDL1 has yet been tested clinically to our knowledge.

Strategies for global cellular PDL1 protein reduction.

Studies of cancer cell PDL1 expression reduction effects on treatment have largely used genetic tumour PDL1 depletion in established cell lines, for example leading to reduced resistance to small-molecule drugs such as mTOR inhibitors[24] or cytotoxic chemotherapy[23]. Aside from genetic PDL1 depletion itself, genetic reduction of gene products promoting PDL1 expression can reduce tumour PDL1 content for therapeutic utility[65,99], and could

selectively reduce cytosolic or nuclear PDL1 based on their mechanisms, as discussed above in Cell-autonomous regulation of cell-intrinsic and subcellular PDL1 content. Genetic approaches are useful for proof of concept but are not practical current clinical strategies. Potential cell-intrinsic PDL1 signal mechanisms that can be or have been therapeutically targeted are shown in FIG. 2.

Several groups have now demonstrated small molecules depleting tumour PDL1 as cancer treatment. Verteporfin, a benzoporphyrin FDA-approved for retinal diseases, reduced cancer cell PDL1 content in distinct human cancer lines and immune and stromal cells through autophagy and inhibition of STAT1 and IRF1-dependent *CD274* (PDL1 gene) transcription. It improved PARP inhibitor efficacy in mouse ovarian tumours in vivo[39]. The natural product curcumin inhibited COP9 signalosome complex 5 protein to deplete cancer cell PDL1 content by promoting its ubiquitination and improved anti-CTLA4 immunotherapy efficacy in mouse 4T1 TNBC[105]. The natural quinolizidine alkaloid product oxymatrine epigenetically reduced PDL1 expression in cultured human SW620 and HCT116 colorectal cell lines and was cytotoxic to them[106]. Aspirin (acetylsalicylic acid) transcriptionally suppressed PDL1 protein and inhibited proliferation of human A549 and H1299 non-small-cell lung cancer cells in vitro[107].

Small-molecule PDL1 and PD1 inhibitors are described[108,109], most of which inhibit surface PDL-PD1 interactions. The subset that reduce cellular PDL1 content could also reduce cancer cell-intrinsic PDL1 signals. Those designed to inhibit PDL1–PD1 surface interactions could also reduce cell-intrinsic PDL1 signals originating from the cell surface. Therapeutically, increasing PDL1 with the cyclin-dependent kinase 4/6 (CDK4/6) inhibitors palbociclib or ribociclib[98], or decreasing it with the EGFR inhibitor gefitinib[54], improved anti-PD1 immunotherapy in mouse models in vivo (for example, 4T1, B16F10, CT26), demonstrating alternative means to control tumour PDL1 for therapeutic benefit. The EGFR inhibitors erlotininb[110] and osemertinib[111] inhibit tumour PDL1 expression, notably in EGFR-mutated human small cell lung cancer lines. Glucose deprivation in vitro or a ketogenic diet in vivo in CT26 tumour-bearing mice reduced tumour PDL1 expression via adenosine monophosphate-activated protein kinase (AMPK)-dependent PDL1 C-terminal tail phosphorylation that reduced PDL1–CMTM4/6 interactions, thereby increasing PDL1 lysosomal targeting for degradation[112]. Metformin (FDA-approved diabetes drug) AMPK dependently phosphorylates PDL1 at S195, leading to subsequent endoplasmic reticulum accumulation and depletion of tumour PDL1, thereby improving anti-CTLA4 immunotherapy efficacy in mouse 4T1 TNBC[113]. In hepatocellular carcinoma, the JAK inhibitor ruxolitinib depleted tumour PDL1 in vitro by preventing IL-6-driven JAK1 phosphorylation at Y112 to stabilize PDL1 (REF.[114]).

The anti-PDL1 antibody H1A prevented PDL1–CMTM6 interaction, thereby redirecting PDL1 to lysosomes for degradation in MDA-MB-231 TNBC cells[34] to prevent cell-intrinsic PDL1-driven radioresistance. In a similar process, a synthetic peptide diverted PDL1 to lysosomes for degradation through an HIP1R-dependent mechanism, in human colorectal cells[79], differing from HIP1R-mediated surface to nuclear PDL1 translocation through PDL1 deacetylation[36]. A peptide inhibitor of PDL1 palmitoylation in the C-terminal cytoplasmic tail or the small molecule 2-bromopalmitate (general palmitoylation inhibitor)

depleted PDL1 by promoting its ubiquitination in vitro and in vivo, respectively, in MC38 mouse colon cancer cells, thereby improving antitumour T cell functions[115]. Neither PDL1 intrinsic signals from specific cancer cell subcellular locations nor PD1 dependence altered by these PDL1 depletion or antagonist agents were described in detail.

Casimersen, eteplirsen and golodirsen are oligonucleotide drugs that alter gene splicing, FDA-approved for Duchenne muscular dystrophy. Similar agents could potentially inhibit pathogenic PDL1 isoforms arising from splicing events while limiting autoimmune side effects. We mined drug libraries to identify 17 candidate PDL1 depletion drugs that depleted tumour PDL1 >2.6-fold in vitro, including in specific subcellular locations that phenocopied genetic PDL1 depletion effects and improved small-molecule DNA damage response inhibitor efficacy in vivo (patent application PCT/US2021/022244). The latter approach is likely the most practical for unbiased identification and rapid clinical translation of PDL1-depleting drugs in humans.

The BET bromodomain inhibitor JQ1 suppressed tumour PDL1 in a mouse model for ovarian cancer and promoted survival through improved antitumour cytotoxic T cell effects[116]. The small molecule tomivosertib inhibits eIF4E phosphorylation and reduced PDL1 expression in a mouse model for hepatocellular carcinoma to reduce tumour growth[117]. None of the foregoing studies of FDA-approved or investigational agents reported effects on specific subcellular PDL1 expression. Much additional work is needed to understand optimal use of these promising strategies. Mechanisms regulating tumour PDL1 expression are extensively reviewed[96,97], whose deep understanding will help to optimize such approaches and identify additional agents to reduce cell-intrinsic PDL1 signalling.

Naturally PDL1-deficient tumours could be more susceptible to IFNα, IFNβ or IFNγ[27], or DNA-damaging cytotoxic agents[23]. Although several studies report that cell-intrinsic tumour PDL1 mediates chemoresistance, there are important exceptions such as tumour PDL1 expression enhancing chemosensitivity in *Braf*^{V600E} mutated colon cancers cells in vitro[25]. We reported that expression of tumour PDL1 positively correlates with autophagy inhibitor susceptibility in melanoma and ovarian cancer[24]. Thus, as depleting PDL1 could increase efficacy of some therapies, it could equally increase resistance to others in distinct cancers. Understanding these cell-intrinsic PDL1 signalling effects will help to inform current treatment regimens involving PDL1 targeting agents and could explain emergence of resistance and/or relapse in specific cancers.

Altering subcellular PDL1 content.

As PDL1 in distinct subcellular locations has differing signal and therapeutic effects, depleting PDL1 in specific subcellular locations could be a better strategy than global PDL1 depletion. Expression of nuclear (but not total) PDL1 in circulating cancer cells in patients with metastatic colorectal or prostate cancer predicted shorter survival versus patients whose circulating cancer cells lacked nuclear PDL1 (REF.[118]). B16 lung metastases exhibited higher nuclear PDL1 versus the primary subcutaneous tumours that expressed predominantly surface membrane PDL1 (REF.[36]). Thus, if nuclear PDL1 contributes to ICB resistance of lung metastases as we predict, a nuclear PDL1 reduction agent could improve efficacy. For example, small-molecule HDAC2 inhibitors (for example, Santacruzamate A)

reduced tumour PDL1 nuclear accumulation and improved anti-PD1 immunotherapy in heterotopic MC38 colon cancer[36]. Additionally, a clathrin-dependent endocytosis inhibitor (pitstop 2) and an inhibitor of importin-α/β-dependent nuclear transport (FDA-approved anti-parasitic agent ivermectin)[36] each reduced PDL1 nuclear translocation in human TNBC MDA-MB-231 cells in vitro[36], although functional consequences were not reported.

Metformin reduces PDL1 protein levels through inducing endoplasmic reticulum accumulation of PDL1 that promoted its degradation[113] and could potentially block specific cell-intrinsic PDL1 signalling that requires processing by the endoplasmic reticulum, but may not affect endoplasmic reticulum-independent pathologic consequences. Endosomal inhibitors could selectively reduce cytoplasmic PDL1 by inhibiting endosomal surface-cytoplasm PDL1 recycling. Identifying other agents trapping PDL1 on the cell surface is warranted. Interestingly, a novel compound (‘drug A’) designed to disrupt PD1–PDL1 surface interaction by inducing PDL1 dimerization and cytosolic uptake reduced growth of heterotopic MC38 colon cancer in vivo[119]. Although this strategy could improve antitumour T cell responses similar to anti-PDL1–PD1 blockade, an unwanted consequence could be paradoxically increased cell-intrinsic PDL1 signalling by intracellular PDL1, driving resistance to certain anticancer agents such as cytotoxic chemotherapy or ICB by non-canonical mechanisms. Thus, a more complete understanding of cell-intrinsic PDL1 signalling from subcellular locations will better inform the design of treatment combinations using novel agents that alter PDL1 cellular distribution. Of note, a report focusing on tumour glucose metabolism using mouse sarcoma, B16 melanoma, L cells and MC38 colon cancer cells incidentally showed that anti-PDL1 antibodies in vitro increased PDL1 in tumour cytosol[95] associated with altered tumour glucose metabolism which could potentially alter cell-intrinsic PDL1 signals, but underlying mechanisms or similar effects using in human cells were not reported.

As discussed above, tumour PDL1 induced by myeloid cell TNF–IL-1β (through NF-κB) versus T cell IFNγ (through STAT1) generated distinct tumour biological phenotypes[104] through unknown mechanisms. We hypothesize that these differences could be from a known differential subcellular PDL1 distribution elicited by the distinct cytokines[35]. Alterations of cytokine-producing cells or agents to affect tumour subcellular PDL1 content could affect cell-intrinsic PDL1 signals through the mechanisms described above and shown in FIG. 2. Specific cellular stressors such as hypoxia increase nuclear PDL1 (REF[35]), implicating selected hypoxia-modulating agents such as bevacizumab[120] as therapeutic cancer cell-intrinsic PDL1 targeting strategies.

Interrupting cell-intrinsic PDL1 signals.

Anti-PDL1 ICB antibodies sensitized mouse B16 melanoma cells directly to IFNβ-mediated cytotoxicity via inhibition of STAT3 in vitro[27]. This report also described clinically occurring somatic *CD274* mutations governing interferon-mediated cytotoxicity sensitivity in human cancers, suggesting clinically relevant cell-intrinsic PDL1 effects that merit investigation. Other reports[23,24,26,78,95], discussed above in the section Cancer cell-intrinsic PDL1 immune effects, suggest that anti-PDL1 ICB antibodies can also alter some cell-intrinsic PDL1 signals, the scope of which remains incompletely defined. Understanding the

additional signalling effects of existing anti-PDL1 (and antibodies inhibiting other immune checkpoints) could lead to new insights into effective treatment combinations.

PDL1 post-translational modifications[53] could be specifically targeted to alter outcomes of these modifications, which was demonstrated using EGFR inhibitors to reduce PDL1 glycosylation, thereby reducing T cell suppression in vitro[54], a strategy that could be used to prevent PDL1 nuclear translocation as glycosylation could promote nuclear PDL1 accumulation[37]. Remarkably, as intracellular PDL1 can bind DNA and globally influence expression of many genes[36], it could regulate gene expression at the epigenetic level and could be explored using techniques such as ATAC-seq of PDL1 replete versus knockout cells. If so, PDL1 transcriptional regulation of gene expression programmes could be altered by available and FDA-approved small-molecule epigenetic inhibitors such as 5-azacytidine.

Finally, work is required to understand how the above cancer cell-intrinsic PDL1 targeting strategies influence antitumour T cell-mediated immunity. As subcellular compartment-specific cancer cell PDL1 mediates such varied immune outcomes, it is likely that some tumour PDL1-depleting strategies will paradoxically improve anti-PDL1 treatments, for example by altering local chemokines or tumour immunogenicity (FIG. 1b; TABLE 1). Some approaches, for example, the HDAC2 inhibitor Santacruzamate A[36], will mediate effects that could alter antitumour immunity. The net clinical effect of cell-intrinsic PDL1 targeting agents will likely require tumour, context and adjunct treatment-specific studies (for example, engineered cytokines and anti-CTLA4).

Conclusions and future considerations

The paradigm of a surface PDL1–PD1 signalling axis is correct but too simplistic and incomplete based on current understanding of the multiplicity of intracellular interactions between PDL1 and many molecules and macromolecular structures governing diverse pathologic processes. This cell-intrinsic PDL1 signalling differs from surface PDL1 signals to PD1 on distinct cells and requires re-thinking of this important paradigm. Likewise, the multiplicity of unexpected cell-intrinsic signal effects of anti-PDL1 antibodies (and likely other ICB antibodies) requires careful reconsideration of their mechanisms of action that can influence patient and combination treatment selection.

Cancer cell-intrinsic PDL1 generates signals affecting growth, survival, metastatic, metabolic, differentiation, stemness and treatment resistance pathways. While mediating biologic mayhem, these pathways also afford novel, actionable treatment targets for potentially more effective interventions or to improve efficacy of existing treatments. We speculate that understanding these cancer cell-intrinsic PDL1 signals could help to explain the incomplete ICB response predicted by expression of tumour PDL1 (REF.[14]), or hyper-progression after ICB[121]. The existence of tumour cell-intrinsic PDL1 signalling thus warrants a more nuanced appreciation of tumour PDL1 status clinically. The remarkable discovery that pathologic cell-intrinsic PDL1 signals can originate from intracellular sources such as the cytoplasm or nucleus implicates PDL1 expression in these locations as useful treatment or prognostic biomarkers requiring much further investigation in humans.

Cancer cell-intrinsic PDL1 signal effects can differ by tumour type, likely owing to mutational landscapes in distinct tumours, variations in individual tumours in patients, tumour microenvironmental factors and differential post-translational PDL1 modifications now recognized to contribute to immune checkpoint efficacy[75] and cell-intrinsic PDL1 signalling[35,36], among other considerations. Additional PDL1 signals arise from disparate sources including exosomes or vesicles bearing PDL1, soluble PDL1 that arises through distinct mechanisms[45], platelets[19], non-hematopoietic cells[22] (BOX 2) and isoforms arising through various mechanisms (FIG. 1a). Assessing cell-intrinsic PDL1 or consequent signalling effects in human clinical trials warrants much additional investigation. A better understanding of the mechanisms directing PDL1 to, or retaining it in, specific subcellular compartments is needed.

Several approaches have already demonstrated the utility of specifically targeting cell-intrinsic PDL1 signals using novel molecules[34,79] or existing research reagents[115]. Repurposing FDA-approved agents is another rapidly translatable approach that has been validated preclinically[24,27,36,39,107], as is using natural products generally regarded as safe, such as curcumin[105] or oxymatrine[106]. Investigational agents also deplete specific subcellular[34,39] and non-surface[20] PDL1 signals. Virtually all of the agents we discuss could suppress cell-intrinsic PDL1 signals in tumour or non-cancer cells. Approaches specifically targeting PDL1 in cancer cells (as opposed to immune or stromal cells) merit further study as off-tumour drug effects might be undesirable in some instances, especially when used in combination with other antitumour agents. Some approaches can be clinically translated rapidly.

Work is needed to place pathologic effects of exosomal, vesicular and soluble PDL1 (REFS[17,18]), including its isoforms[17,40-45] (BOX 2; FIG. 1a), in context relative to effects of cancer cell-intrinsic and cell-extrinsic PDL1 signals, which also requires study in distinct tumours. For example, PDL1 regulation of local chemokines will affect tumour-infiltrating lymphocytes known to alter ICB. PDL1 control of the DNA damage response[34] or DNA damage[32] could suppress tumour immunogenicity and PDL1 reduction could increase tumour immunogenicity from increased cytosolic nucleic acid activation of cGAS-STING[122]. PDL1 control of pyroptosis[35] or apoptosis[29] could likewise be actionable.

In some instances, cancer cell-intrinsic PDL1 signals are beneficial to treatment. For example, genetic PDL1 depletion suppresses tumour sensitivity to small-molecule autophagy inhibitors[24] or selected cytotoxic agents[25]. These effects are tumour-specific, which likely relates to tumour-specific mutations, tissues or origin, the local microenvironment and other factors. Differentiating such detrimental versus beneficial effects of targeting cell-intrinsic PDL1 is an important goal.

Some PDL1 biology merits additional study for translational potential. For example, the cell cycle dependence of PDL1 expression suggests that combining cytotoxic agents with anti-PDL1 could be improved using cytotoxic agents specifically active in the cell cycle phase when PDL1 expression peaks, such as gemcitabine[123]. Agents active across the entire cell cycle might be less effectively combined, such as taxanes[124] and platinum drugs[125]. These distinctions are important in that taxanes and platinum agents are in FDA-approved anti-

PDL1 (and anti-PD1) combinations. Immune cell-intrinsic PDL1 signals could alter tumour immune surveillance or treatment responses but are little studied. There are no reported effects of altering cancer cell-intrinsic PDL1 signals in clinical trials, or of testing specific subcellular PDL1 expression levels as a treatment response biomarker to our knowledge.

PDL1 is not unique in its potential for pathologic cancer cell-intrinsic signals. It is an immunoglobulin superfamily member whose other members thus far studied share signalling similarities with PDL1, although this aspect is as yet little studied. A recent report that tumour microenvironmental IgA mediates cell-intrinsic tumour signals[126] suggests that immunoglobulins themselves mediate important cell-intrinsic signals, but no known immune checkpoint molecule has been shown to mediate cell-intrinsic signalling from its immunoglobulin structure per se. Cell-intrinsic signal effects of other immunoglobulin superfamily members (BOX 2) is an area warranting additional investigation.

Essentially, all in vivo tumour PDL1 biology studies have been done in established tumours. Whether PDL1 specifically affects carcinogenesis and whether cell-intrinsic PDL1 signal effects differ in tumours arising from PDL1 null versus PDL1-expressing cells of origin have yet to be reported but are important to understand. Such studies would require the development of cancer cell of origin (for example, melanocyte) specific *Cd274*-knockout mouse models.

PDL1 depletion could combine well with small-molecule DNA damage response inhibitors such as PARP inhibitors known to augment cGAS–STING-driven immunity[127,128] but also with agents inhibiting other DNA damage response pathways. As PDL1 can also directly bind to DNA[36] or RNA[34], cell-intrinsic PDL1 could theoretically regulate immunogenicity of nucleic acids themselves and their recognition by innate immune sensing pathways not limited to cGAS–STING (for example, Toll-like receptors-retinoic acid-inducible gene 1–mitochondrial antiviral signalling protein)[129] or transcription and/or stabilization of mRNAs encoding immune stimulatory effectors. As PDL1 depletion in cancer cells alters immune cell trafficking chemokines[91] and could engender tumour immunogenicity[35], we expect PDL1 depletion could improve efficacy of immunotherapies, including FDA-approved anti-PDL1 or anti-PD1 ICB antibodies, despite reduced tumour PDL1 expression, and other modalities known to benefit from antitumour immunity such as cytotoxic chemotherapy[130]. Of note, specific DNA damage such as double-strand DNA breaks induce PDL1 expression in an ataxia telangiectasia and Rad3-related ATR-Checkpoint kinase 1-dependent mechanism[131,132]. Use of selective small-molecule inhibitors of the ATR–ataxia telangiectasia mutated (ATM) pathway could prevent DNA damage-induced PDL1 expression resulting in simultaneous suppression of intrinsic and extrinsic PDL1 signals.

These are interesting times for the development of advanced cancer immunotherapies and rational combinatory approaches to improve the outcomes of patients with cancer. We expect that the targeting of cancer cell-intrinsic PDL1 signals will prove useful in this arena.

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Box 1|

Cell-extrinsic versus cell-intrinsic programmed death ligand 1 signalling mechanisms

Current immune checkpoint blockade (ICB) dogma states that tumour programmed death ligand 1 (PDL1) is expressed on the surface of cancer or immune cells and is pathogenic primarily by inhibiting programmed death 1 (PD1)-expressing $CD8^+$ antitumour T cells[3,6,10-15]. However, the multiverse of tumour PDL1 signalling extends beyond cell-extrinsic PDL1 signalling to immune cells to include important cell-intrinsic effects. We define 'cell-extrinsic' PDL1 signalling as any signal mediated by surface PDL1 outside the PDL1-expressing cell to alter signalling in adjacent tumour or non-tumour cells. Specifically, such signals include canonical cell-extrinsic PDL1 engagement with PD1-expressing immune cells and downstream signalling in immune cells. Adjacent cancer cells can also express PD1, or as yet little studied tumour surface PDL1 receptors (for example, CD80 (REF.[73]) and integrins[76]), resulting in cell-extrinsic PDL1 surface signalling-driven cancer cell-intrinsic consequences. By contrast, we define cancer 'cell-intrinsic' PDL1 signalling as any PDL1-driven signal altering biology within, or inside, that PDL1-expressing cancer cell. For example, PD1 engagement with cancer cell surface PDL1 could elicit PDL1 reverse signalling as now seen in immune cells[68,77,134,135]. Thus, PDL1 and PD1 could be both ligands and receptors for each other in a non-mutually exclusive manner. Although CD80-mediated surface PDL1 reverse signalling might be possible, it has not been described in the literature. Cancer cell surface PDL1 can also transduce cell-intrinsic signals independent of PD1 ligation[27] but mechanistic underpinnings are incompletely defined. Surface PDL1 could also signal intrinsically by acting as a co-receptor for other cancer cell surface receptors as suggested by reports of integrin[33] and (weak) extracellular growth factor receptor (EGFR)[137] interactions in human bladder and lung cancer cells, respectively, but more work is required to understand the functional significance and generalizability of data. Remarkably, cell-intrinsic PDL1 signals can also be transmitted by fully intracellular PDL1, independent of any PD1 engagement, through macromolecular interactions. Intracellular PDL1 (for example, nuclear or cytosolic) participates in specific protein–protein[37-38] and protein–nucleic acid[34-36] interactions to regulate cell-intrinsic tumour pathogenic signals (FIG. 1b; TABLE 1). The US Food and Drug Administration (FDA) approved anti-PD1 or anti-PDL1 ICB antibodies were designed to disrupt surface PDL1–PD1 interactions specifically. Based on mechanistic work on tumour PDL1 signalling so far, many pathogenic cell-intrinsic PDL1 effects are refractory to inhibition by currently available ICB agents, thus warranting the need for additional tumour PDL1 targeting strategies or use of cell-intrinsic PDL1 effects as treatment response biomarkers.

Box 2 |

PDL1 from extracellular PDL1 and non-cancer cells, and related molecules

Non-cancer cells and exosomes

Aside from tumour cells, programmed death ligand 1 (PDL1) signals on non-tumour cells and structures can mediate homeostatic and/or pathologic outcomes. For instance, endothelial cell PDL1 generates regulatory T cells[21] that can inhibit antitumour or autoimmune responses. PDL1-deficient versus control conventional dendritic cells intrinsically produce more inflammatory cytokines in tumours[70]. In the non-tumour context, cell-intrinsic PDL1 signals in dermal dendritic cells can promote their migration to draining lymph nodes and subsequent activation of T cells by enhancing signalling downstream of chemokine receptors (for example, ERKs and F-actin polymerization)[138]. T cell-intrinsic PDL1 inhibits $CD4^+$ T cell activation by activating signal transducer and activator of transcription 3 (STAT3)[68]. Natural killer cell PDL1 can improve the cells' antitumour cytotoxicity through a PD1-independent, p38 mitogen-activated protein kinase (MAPK)-dependent pathway[67]. PDL1 promotes pyroptosis in human pulmonary arterial smooth muscle cells under hypoxia that augments pulmonary vascular fibrosis[69]. PDL1 presented by platelets can suppress antitumour immunity in mouse heterotopic MC38 colon cancer and various human PDL1-negative cancer lines in vitro[19]. Both haematopoietic (including immune cell) and non-haematopoietic PDL1 contribute to virus defence[22]. We anticipate that cell-intrinsic PDL1 signals from immune and non-immune cells can contribute to anticancer immunity, an area deserving additional studies. X-irradiated *Cd274*-knockout mice survive a significantly shorter time versus X-irradiated wild-type mice, an effect the authors attributed to defective DNA damage repair[34] but which could be multifactorial, and is nonetheless consistent with cell-intrinsic PDL1 effects in normal cells. Some non-cancer cell PDL1 signals appear to overlap functionally with cell-intrinsic PDL1 signals in cancer cells (TABLE 1). The role of cell-intrinsic PDL1 signals in normal cells under homeostatic conditions or during cellular transformation requires much further investigation. We reported that adipocyte PDL1 suppresses response to immune checkpoint blockade (ICB) and the peroxisome proliferator-activated receptor-γ (PPARγ) antagonist GW9662 can selectively reduce adipocyte PDL1 to improve ICB in murine models of breast cancer[20], demonstrating a proof of concept for pharmacologic cell-type selective, cell-intrinsic PDL1 targeting. Details of exosome biology and exosomal PDL1 (REF.[17]) and effects of soluble PDL1 on cancer immunotherapy[18] were recently comprehensively reviewed.

Other immunoglobulin superfamily members

PDL1 is an immunoglobulin superfamily member. Interestingly, other immunoglobulin superfamily members were recently reported to mediate cancer cell-intrinsic signals, some similar to PDL1 signals in various mouse and human cancers including in vivo effects on distinct treatments. These immunoglobulin superfamily members include programmed death 1 (PD1) (REFS[23,24,71,72,139]), PDL2 (the other PD1 ligand)[73,140,141], CD80 (the other PDL1 receptor)[73], B7-H3 (REFS[142-145]), CD90 (REFS[146,147]), AXL[148] and IgA itself[126]. Fat mass and obesity-associated protein promotes PD1 expression

associated with increased mTOR in human MDA-MB-231 and BT549 cells, and mouse 4T1 TNBC cells, but a mechanistic relation to PDL1–PD1 signals was not demonstrated[149]. PDL1-independent mTOR promotion from PD1 on colon cancer and human hepatoma cells in vitro was reported[150]. Myeloid cell PD1 inhibits their phagocytosis[151] and T cell-intrinsic V-type immunoglobulin domain-containing suppressor of T cell activation (VISTA) promotes T cell quiescence (R. Noelle, personal communication).

Box 3 |

Effects from the programmed death ligand 1 C-terminal tail

Programmed death ligand 1 C-terminal tail signals

The short (30 amino acid) human programmed death ligand 1 (PDL1) carboxy-terminal tail lacks canonical signalling motifs and is the least structured PDL1 domain. Yet, it binds nucleic acids to promote mRNA stability[34]; binds specific DNA promoter regions to enhance gene expression[36]; has a hypoxia-mediated p-STAT3 binding site that promotes PDL1 nuclear translocation and gasdermin C-driven pyoptosis[35]; has an acetylation site, acetylation of which inhibits nuclear PDL1 translocation[36]; has a lysosome-targeting motif to promote its degradation[79]; contains palmitoylation[115] and phosphorylation[112] sites that inhibit its ubiquitination; promotes genomic stability[38]; and promotes immune checkpoint blockade (ICB) resistance through other distinct mechanisms[27,28,36]. Most of these properties have not yet been reported for mouse PDL1 but are likely given structural similarities to human PDL1 (REF.[56]).

The mouse C-terminal tail has defined motifs that overall inhibit interferon-β (IFNβ)-mediated cell cytotoxicity, and also inhibit signal transducer and activator of transcription (STAT3), which could contribute to cell-intrinsic resistance to type I and II interferons[27]. In this report, genetic PDL1 silencing increased interferon-mediated tumour cytotoxicity through enhanced caspase 7 activity, although caspases 3 and 9 were also inhibited by PDL1. Additionally, this report did not distinguish cell-intrinsic effects on IFNβ signalling mediated by intracellular (cytosolic or nuclear) or surface PDL1. Specific mechanisms for mouse PDL1 C-terminal tail regulation of these outcomes (for example, caspase expression) and PDL1-driven STAT3-mediated interferon sensitivity in human cells is possible but as yet unreported.

Cell-intrinsic PDL1 effects not mediated by the C-terminal tail

PDL1 signals other than from its C-terminal tail are, thus far, only reported in human cells. A tail-deleted PDL1 secreted isoform can mediate immune suppression[59], discussed above.PDL1 isoform C lacking the C-terminal tail and transmembrane domain promoted colorectal cancer cell proliferation and epithelial–mesenchymal transition[43]. A report of non-C-terminal tail PDL1 signals provided some evidence that protein kinase B (PKB; also known as AKT) and F-actin accumulation are regulated by PDL1 amino acids 128–237 (REF[83]), essentially spanning the Ig-C domain, far amino-terminal to the C-terminal tail. However, functional tests of this PDL1 moiety were not demonstrated. By contrast, functional studies of a YSR-like motif in the PDL1 N terminus at the Ig-V/Ig-C domain boundaries abrogated nuclear PDL1 stabilization of the cohesin complex, but not its PD1 binding ability, producing defective sister chromatid cohesion[37], suggesting properties of cell-intrinsic PDL1 independent of its C-terminal tail. Additional signal functions of non-tail PDL1 moieties remain to be elucidated in detail, including in mouse cells.

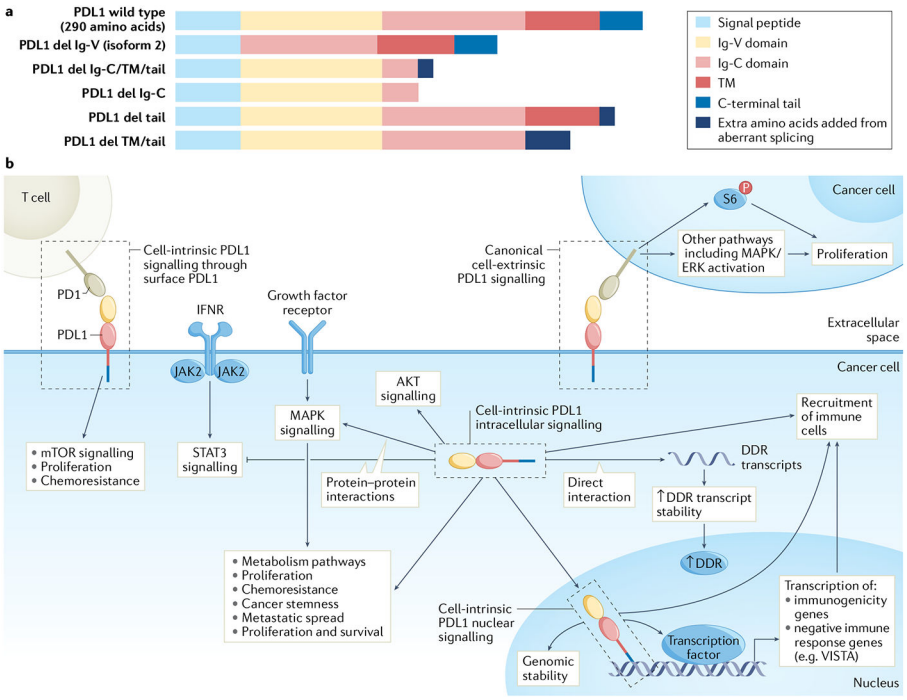

Fig. 1 |. Programmed death ligand 1 signalling in cancer cells.

a | Programmed death ligand 1 (PDL1) comprises several domains, the order and presence of which differ between wild-type PDL1 and its isoforms. PDL1 isoforms can be secreted by loss of transmembrane anchoring through splicing or retained in subcellular compartments where they could control cell-intrinsic signals, all requiring further investigation. PDL1 isoforms aside from full-length (isoform 1) PDL1 or its enzymatic cleavage product in vesicles, exosomes or soluble form are not described, **b** | Surface PDL1 exhibits immunoglobulin-like topology with amino-terminal extracellular structure (yellow and light red rectangles) attached to a transmembrane (TM) domain (dark red rectangle) and a short carboxy-terminal tail (blue rectangle). The immunoglobulin-like extracellular domain interacts with the immunoglobulin-like domain of extracellular programmed death 1 (PD1). Cell-intrinsic full-length intracellular PDL1 (for example, cytoplasmic or nuclear) has same sequence as PDL1 expressed on the cell surface, but its N-terminal domain is likely unfolded and, thus, could interact with proteins distinctly from surface-expressed PDL1, and likely cannot engage PD1 using extracellular topology. Surface PDL1 appears capable of reverse signalling through its cell-intrinsic C-terminal tail (cell-intrinsic PDL1 surface signalling, or reverse PDL1 signalling), requiring additional mechanistic studies to confirm, or could signal to adjacent cancer cells expressing PD1 (cell-extrinsic PDL1 signalling), CD80 or other receptors such as specific integrins. PDL1 in cytosol can directly interact with resident molecules, for example, mRNA and others described in text, or in nucleus to act as a co-transcription factor, or mediate other effects described in text. Tumour surface PDL1 signalling to PD1 on distinct cancer cells (cell-extrinsic PDL1 surface signalling) can promote cancer cell proliferation, for example by increasing phosphorylation of ribosomal S6 protein (p-S6) in PD1-expressing cancer cells among other effects. Cytosolic PDL1 can control mRNA stability to regulate expression of genes involved in the DNA damage response (DDR) or could directly promote major signalling pathways

such as mitogen-activated protein kinase (MAPK)/extracellular-signal-related kinase (ERK) or signal transducer and activator of transcription 3 (STAT3) through protein–protein interactions. Nuclear PDL1 exerts transcriptional control of specific target genes involved in tumour immunogenicity or can have non-transcriptional effects on chromosome stability through sister chromatid. These cell-intrinsic PDL1 signals affect antitumour immune responses distinctly from surface PDL1. del, deletion; IFNR, interferon receptor; VISTA, V-type immunoglobulin domain-containing suppressor of T cell activation.

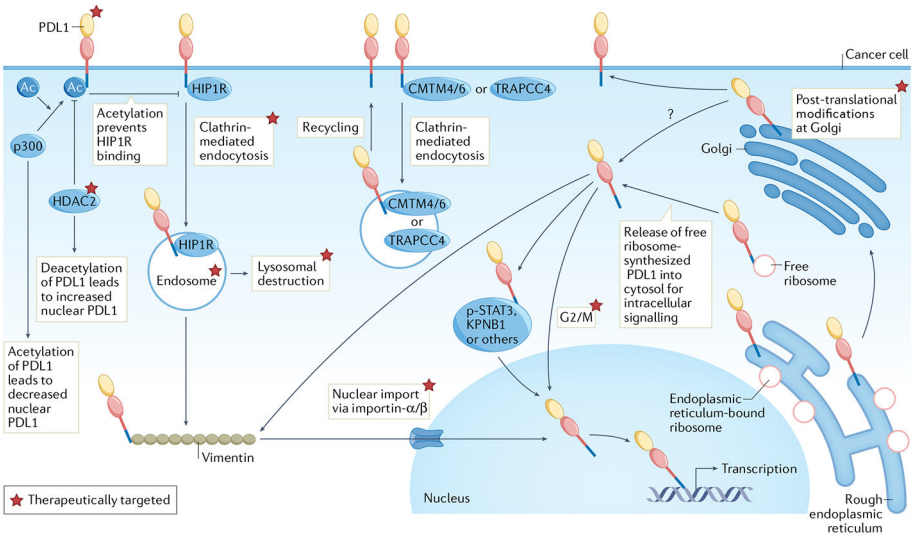

Fig. 2 |. Regulation of intracellular PDL1 and potential targeting.

Programmed death ligand 1 (PDL1) is synthesized in endoplasmic reticulum followed by Golgi processing and surface presentation. Huntingtin-interacting protein 1-related protein (HIP1R) targets PDL1 for lysosomal destruction whereas CKLF-like MARVEL transmembrane domain-containing protein 4/6 (CMTM4/6) or trafficking protein particle complex subunit 4 (TRAPPC4) recycles endocytic PDL1 from cytoplasm to surface. HIP1R-dependent clathrin-mediated endocytosis of surface, non-acetylated PDL1 (controlled by histone deacetylase 2 (HDAC2) and acetyltransferase p300) facilitates PDL1 (from free ribosomes) interaction with vimentin followed by nuclear import via importin-α/β. We show p300 and HDAC2 as acting on cell surface PDL1, but those interactions could also occur in cytosol or nucleus. PDL1 can interact with p-STAT3 in triple-negative breast cancer (TNBC) during hypoxia or associate with karyopherin B1 (KPNB1) in lung cancer, either pathway increasing nuclear PDL1. Free ribosomes (that is, not endoplasmic reticulum-bound) can theoretically synthesize PDL1 for direct release into the cytosol without modification in the endoplasmic reticulum and Golgi. Thus, the cellular source of nuclear PDL1 could be from the surface or cytosol, or directly from other organelles following PDL1 processing such as endoplasmic reticulum/Golgi. PDL1 can participate in macromolecular interactions, for example, the endoplasmic reticulum-associated protein degradation pathway, but whether endoplasmic reticulum-bound PDL1 mediates cell-intrinsic signalling is unknown. Apart from acetylation, phosphorylation, glycosylation, palmitoylation and polyubiquitination, other post-translational PDL1 modifications regulate its subcellular distribution, but mechanistic details are not comprehensively characterized. Exogenous ligands and cellular stressors can upregulate PDL1 expression or redistribute surface PDL1 to other locations including the cytosol and nucleus or into organelles such as lysosomes or autophagosomes. Examples include tumour necrosis factor (TNF), interferons, DNA damage (from exogenous sources such as cytotoxic chemotherapy) or environmental cues such as hypoxia or nutrient (for example, glucose) availability. PDL1 cell-intrinsic trafficking is significantly controlled by the cell cycle, decreasing in the G1 phase and increasing in S/G2/M cycles. Regulation of PDL1 cell-intrinsic signalling directly from mitochondria, endoplasmic reticulum, autophagosomes or other non-nuclear organelles remains to be elucidated.

Altering cell-intrinsic PDL1 content or signalling apart from surface PD1–PDL1 signal disruption using US Food and Drug Administration (FDA)-approved antibodies elicits distinct therapeutic vulnerabilities. Total tumour PDL1 can be reduced using approaches including agents targeting PDL1 post-translational modifications such as palmitoylation and glycosylation (for example, peptide mimics, glutathione synthase kinase 3β (GSK3β) inhibitors), antibodies disrupting PDL1–CMTM4 or CMTM6 interactions (for example, H1A), repurposed FDA-approved drugs (for example, the photosensitizer verteporfin) and natural products (for example, curcumin). Subcellular PDL1 redistribution can be altered using agents disrupting clathrin-mediated endocytosis (for example, pitstop 2), macromolecular interactions (for example, cytoskeleton or signal transducer and activator of transcription 3 (STAT3) inhibitors) and importin-α/β molecules for nuclear import (for example, FDA-approved ivermectin). Therapies sequestering PDL1 on cell surface or in other compartments such as endosomes or autophagosomes include FDA-approved cell-cycle inhibitors (for example, palbociclib, ribociclib), endocytic inhibitors (for example, Dyngo-4a) and autophagy inhibitors (for example, hydroxychloroquine).

Table 1|

Programmed death ligand 1 signal outcomes and mechanisms

Cancer cell PDL1 signal	Subcellular PDL1 location	Functional consequence	Mechanisms	Tumour types	Experimental models and context
Intrinsic	Surface	Increase myeloid-derived suppressor cell recruitment and anti-PD1 resistance	$CD8^+$T cell IFNγ induces cancer cell PDL1 signalling to promote STAT3-dependent, cell-intrinsic NLRP3 inflammasome activation	Melanoma	In vivo mouse models[28]
Intrinsic	Unknown, but localized to carboxy-terminal tail (RMLDVEKC motif)	Inhibits STAT3 activation	Inhibits IFNα, 1FNβ and IFNγ signals and sensitivity in mouse cells	Melanoma	In vitro studies of mouse cell lines[27]
Intrinsic	Surface	Anti-PDL1 antibodies sensitized mouse B16 melanoma cells directly to IFNβ-mediated cytotoxicity in vitro	Unknown	Melanoma, colon, breast	In vitro studies of mouse cell lines[27]
Intrinsic	Surface	PDL1 suppresses FAS-mediated apoptosis	Unknown but involves PD1-induced, tumour surface PDL1 back-signalling	P815 mastocytoma	In vitro studies of mouse cell lines[29]
Intrinsic	Surface	Promote SNAIL protein stability and immune-independent metastasis	Antagonize PTP1B, promote MAPK signalling	Breast	In vitro and in vivo human[79] and mouse[79] models
Intrinsic	Intracellular/ perinuclear	Increase tumour resistance to chemotherapy	Enhance DNA-PK-mediated RAS–MAPK activation	Breast	In vitro studies of human cell lines[81]
Intrinsic	Surface	Increase tumour susceptibility to chemotherapy	Promote expression of pro-apoptotic proteins BIM and BIK	Colon (*BRAF*^{V600E})	In vitro and in vivo human[25] and mouse[25]
Intrinsic	Nuclear	Anti-PD1 resistance	Potentially by mediating transcription of immune checkpoint ligands (e.g. VISTA, galectin 9)	Heterotopic colon cancer	In vivo mouse model[36]
Intrinsic	Nuclear	Possibly regulate tumour immunogenicity through hypoxia-mediated pyroptosis	Activate gasdermin C to induce tumour necrosis and tumour growth promoting inflammation	Breast	In vitro studies with human[35] cell lines and in vivo mouse models[35]
Intrinsic	Nuclear	Increase cell proliferation by enhancing GAS6–MERTK signalling	Nuclear PDL1 binds to SP1 transcription factor to increase *Gas6* gene transcription	Non-small-cell lung cancer	In vitro and in vivo studies of human cell lines[80]
Intrinsic	Unknown	Renders tumour immune cytolytic-resistant	Unknown, related to anti-apoptosis?	Melanoma	In vitro experiments of a syngeneic mouse cell line[28]
Intrinsic	Unknown	Regulates MHC class I expression possibly affecting antigen presentation	Controls transcription of immune response genes	Breast	In vitro studies of human cell lines[36,91]
Intrinsic	Cytosol	Cancer cell-intrinsic PDL1 regulates expression of genes involved in the DNA damage response	Cytoplasmic PDL1 binds to specific mRNAs with a GAAGAA/U motif to outcompete the RNA exosome	Breast, colon	In vitro studies of human cell lines[34]

Cancer cell PDL1 signal	Subcellular PDL1 location	Functional consequence	Mechanisms	Tumour types	Experimental models and context
Intrinsic	Unknown	Could alter chemokines/TIL trafficking	Controls tumour NF-κB-mediated transcription (e.g. binds to RelA)	Breast, colon	In vitro studies of mouse[91] and human[36] cell lines
Intrinsic	Unknown	Alter autophagic flux and response to autophagy inhibitors	Could affect antigen processing	Melanoma, bladder	In vitro studies of mouse[23,24] and human[23,24] cell lines
Intrinsic	Unknown	Promote tumour stemness	mTORC1-driven *Oct4* gene expression	Melanoma, ovarian	In vitro and in vivo studies of mouse cell lines[133]
Intrinsic	Unknown	Increase tumour glucose metabolism	Possibly allow tumours to outcompete T cells for glucose, causing T cell dysfunction by driving mTORC1 activation	Sarcoma, B16 melanoma, L cells, MC38 colon	In vitro studies of a mouse cell line[95]
Intrinsic	Unknown	Increase tumour metastases	Reduce immunotherapy efficacy due to metastases	B16 melanoma, breast	In vivo studies of mouse[24] and human[85] models
Intrinsic	Nuclear	Promote genomic stability	Facilitate cohesin complex formation by binding cohesin subunit SA1	Rectal	In vitro studies of human[38] cell lines
Intrinsic	Nuclear	Promote sister chromatid cohesion through amino-terminal YSR-like motif	Facilitate cohesin complex retention on chromatin	TNBC	In vitro studies of human cell lines[37]
Intrinsic, isoform	Unknown	Epithelial–mesenchymal transition signature	Activating PI3K–AKT signals; GSK3β-mediated phosphorylation, ubiquitination and degradation of SNAIL; RAS–ERK activation	Colorectal, glioblastoma, non-small-cell lung cancer, nasopharyngeal, oesophageal, TNBC	In vitro studies of human[31,84,85,89] and rat[84] models
Extrinsic	Surface	Reduce cell growth rate and possibly drive anti-PD1/PDL1-associated tumour growth	Tumour surface PDL1 engages tumour surface PD1 to suppress p-AKT and/or p-ERK activation	Non-small-cell lung cancer	In vitro and in vivo study of human[72] cell lines/xenografts
Extrinsic	Surface	Increase cell growth rate and possibly explain anti-PD1/ PDL1 treatment response	Tumour surface PDL1 engages tumour surface PD1 to increase via p-S6 signals	Melanoma	In vitro and in vivo studies of mouse[71] and human[71] models; IHC from patient samples
Extrinsic	Surface	Inhibit PD1-expressing antitumour immune cells, especially T cells	Direct immune cell engagement with PDL1 back-signalling	Lung, ovary, colon, melanoma, mastocytoma, myeloma, non-small-cell lung cancer, renal cell, pancreatic, gastric, breast and non-tumour models	In vitro and in vivo human and mouse models[3-12,14,15]
Extrinsic	Surface	Prevent T cell co-stimulation	Interacting with CD80 in *cis* or in trans	Non-tumour models	In vivo mouse models[51,52]
Extrinsic	Surface	Induce T cell anergy, exhaustion and/or death	Direct immune cell engagement with back-signalling	Non-tumour models	In vivo human and mouse studies[68,134,135]
Extrinsic	Surface	Inhibit T cell memory	Reduce effector memory, T cell stem cell and resident T cell memory through unclear mechanisms	Melanoma, non-small-cell lung cancer	In vivo human[136] and mouse[136] studies

BIK, BCL-2-interacting killer; BIM, BCL-2-like protein 11; DNA-PK, DNA-dependent protein kinase; ERK, extracellular-signal-related kinase; IFNγ, interferon-γ; GSK3β, glutathione synthase kinase 3β; IHC, immunohistochemistry; MAPK, mitogen-activated protein kinase; MERTK, tyrosine-protein kinase MER; MHC, major histocompatibility complex; mTORC1, mammalian target of rapamycin complex 1; NF-κB, nuclear factor-κB; NLRP3, NOD-, LRR- and pyrin domain-containing 3; PD1, programmed death 1; PDL1, programmed death ligand 1; p-S6, phospho-S6 ribosomal protein; PTP1B,

protein-tyrosine phosphatase 1B; RelA, REL-associated protein; SA1, cohesion subunit SA1; SP1, specificity protein 1; STAT3, signal transducer and activator of transcription 3; TIL, tumour infiltrating lymphocyte; TNBC, triple-negative breast cancer; VISTA, V-type immunoglobulin domain-containing suppressor of T cell activation.