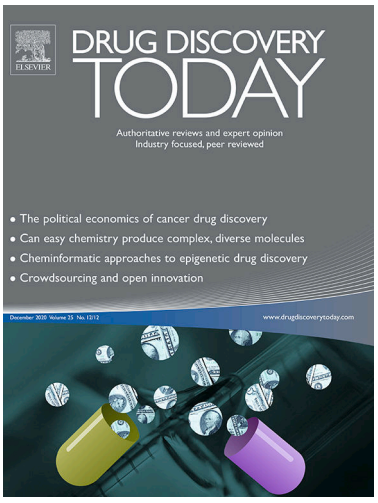
Journal Pre-proofs



Post-screen (grey)

PD-L1 small-molecule modulators: a new hope in epigenetic-based multidrug cancer therapy?

Clemens Zwergel, Rossella Fioravanti, Antonello Mai

PII: S1359-6446(22)00428-7
DOI: https://doi.org/10.1016/j.drudis.2022.103435
Reference: DRUDIS 103435

To appear in: *Drug Discovery Today*

Received Date: 29 April 2022
Revised Date: 31 October 2022
Accepted Date: 7 November 2022

Please cite this article as: C. Zwergel, R. Fioravanti, A. Mai, PD-L1 small-molecule modulators: a new hope in epigenetic-based multidrug cancer therapy?, *Drug Discovery Today* (2022), doi: https://doi.org/10.1016/j.drudis.2022.103435

PD-L1 small-molecule modulators: a new hope in epigenetic-based multidrug cancer therapy?

Clemens Zwergel[1], Rossella Fioravanti[1], and Antonello Mai[1,2]

[1]Department of Drug Chemistry and Technologies, Department of Excellence 2018-2022, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
[2]Pasteur Institute, Cenci-Bolognetti Foundation, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy
Corresponding authors: Zwergel, C. (clemens.zwergel@uniroma1.it) ; Mai, A. (antonello.mai@uniroma1)
Keywords: immunotherapy; PD-L1; epigenetics; cancer; therapy escape; multitarget approach.
Teaser: This article reviews the latest findings regarding PD-L1 immunotherapy related to epigenetically influenced pathophysiological conditions and the state of the art in drug design of PD-L1-targeting agents and combinatory therapies.

Programmed death-ligand 1 (PD-L1) is an immune checkpoint protein the overexpression of which results in an inhibitory signal that induces T cell exhaustion responsible for immune escape in tumors. Immunotherapy strategies targeting the PD-L1 pathway have achieved remarkable success in treating various types of cancer. More recently, numerous advances in understanding the complex PD-L1 biology have been made, and the first small-molecule inhibitors have been described in the literature. In this review, we highlight the most promising recent advances in understanding the complex regulation mechanisms focusing on small-molecule modulators, which could be used in rational therapy combinations with other epigenetic chemotherapeutic agents.

Introduction

Over the past few years, there has been significant progress in cancer immunotherapy[1] and immune checkpoints are now considered key players in cancer biology because tumor cells are able to evade immune surveillance by distinct immune checkpoint pathways.[2] PD-L1, also known as cluster of differentiation 279 (CD279), acts as an immune checkpoint by negatively regulating T cells via the suppression of the PI3K/AKT and Ras/MAPK/ERK signaling pathways.[3,4] PD-L1 is expressed mainly in peripheral tissues and the tumor microenvironment (TME).[5,6] Activation of the PD-L1 signaling pathway is crucial for the regulation of T cell activation, proliferation, and apoptosis, thus having a direct influence on the cell-mediated immune response.[7] PD-L1 is often highly expressed in tumor-infiltrating lymphocytes, B cells, natural killer (NK) cells, monocytes, and dendritic cells (DCs), and its overexpression results in an inhibitory signal that induces T cell exhaustion,[8] responsible for immune escape in tumors.[9] Thus, PD-L1 acts as a type of tumor cell protector, shielding them from T cell-mediated elimination.[10] Conversely, under physiological conditions, PD-L1 is responsible for immune tolerance, maintenance of immune homeostasis, and protection from excessive autoimmune damage to healthy tissues.[10]

Immunotherapy strategies targeting the PD-L1 pathway have been successfully used to treat numerous types of cancer.[1,11] The PD-L1/PD-1 axis has a fundamental role in escaping the immune system in the advanced stages of Hodgkin's lymphoma,[12] nonsmall-cell lung cancer (NSCLC),[13] renal cancer, urothelial carcinoma,[14] and melanoma.[15] This implies that immune checkpoint inhibitors (ICIs), capable of blocking the activity of immune checkpoints, could result in the release of the immune brake, reactivation of the immune response of T cells to the tumor cells, and finally, antitumor effects.[16] More than 1000 clinical trials have evaluated anti-PD-1/anti-PD-L1 antibodies, and some of which, such as Keytruda (pembrolizumab), Opdivo (nivolumab), Libtayo (cemiplimab), Tecentrip (atezolizumab), Bavencio (avelumab), and Imfinzi (durvalumab), have been approved by the US Food and Drug Administration (FDA) for more than ten cancer indications.[12]

Following considerable advances in the characterization of PD-L1 biology, numerous small molecules have appeared in both patents and in the literature(reviewed in [17–19]). In principle, small molecules are preferred over antibodies because they have significant advantages, such as stability, membrane permeability, non-immunogenicity, and the possibility of oral administration.[20] Their main drawback is that most compounds follow a limited pharmacophore containing a biphenyl structure. In addition, relatively little is known regarding their pharmacological modulation via small molecules[21]; most small molecules have only been reported as patents, with relatively few data generally available,[19,22] and even fewer compounds having reached clinical trials.[19,22] The most advanced compounds are CA-170 (Phase I completed for solid tumors or lymphomas, no data yet available), GS-4224 or Evixapodlin (Phase I completed, with promising data in patients with solid tumors, Phase II ongoing, NCT04049617) and INCB086550 (Phase I preliminary promising data published, Phase II ongoing in patients with solid tumors) with partially promising data available.[23–25]

Tumors and immune systems in patients are very diverse; blocking of PD-L1 still has slow and low (20–40%) response rates, indirectly controlling cancer growth.[26] Recent published evidence underlined that, for an efficient anti-PD-L1 therapy, an intact

integrated immune cycle needs to be established, because damage in any step of this cycle often leads to therapy failure .[27] This cycle has three crucial steps: (i) cancer-antigen presentation and T cell activation; (ii) trafficking of T cells to tumors; and (iii) T cell-mediated recognition and elimination of tumor cells.[28]

There is growing evidence that an impaired immune cycle can be rescued by other chemotherapeutic agents, especially epigenetic modulators; thus, a rational combination or a hybrid approach of PD-L1 modulation with modulators of epitargets could improve the success of checkpoint blockade therapies in the clinic.[11]

Epigenetic modifications include DNA methylation, histone modification, and RNA modifications. Aberrant epigenetic patterns have been associated with the onset and development of many types of cancer.[29] Numerous epimodulators, such as inhibitors of histone deacetylases (HDACs), DNA methyltransferases (DNMTs), lysine-specific demethylase 1 (LSD1), bromodomains, or enhancer of zeste homolog 2 (EZH2) (Figure 2), have been developed in preclinical and clinical settings based on the growing understanding of the plethora of epigenetic mechanisms.[30–32] Epigenetic patterns are altered not only in cancer cells, but also in immune cells.[33] This dysregulation is likely to be crucial for the immunogenic deficiency of cancerous cells and the subsequent augmentation of more immunosuppressive cells.[34] Epigenetic modifications can activate silent genes, which can be immune checkpoint regulators that either augment the immune response or lead to immune evasion.[35] As outlined for PD-L1, monotherapy outcomes with epidrugs were satisfactory in hematological malignancies, whereas solid tumors often showed unsatisfactory results.

Importantly, there is increasing evidence that epigenetic modulators can influence each step of the immune cycle, from antigen presentation and T cell regulation to the modulation of the immunosuppression of cancerous cells.[36] For example, cytokines can be epigenetically downregulated, leading to lower infiltration rates of T lymphocytes; at the same time, endothelin receptors are overexpressed, resulting in cancer progression and angiogenesis.[27]

Strikingly, there are currently numerous clinical trials involving PD-L1 antibodies associated with epigenetic modulators (see [37] for details). In this review, we critically discuss recent findings to stimulate researchers to develop novel potential therapeutic concepts using PD-L1 inhibitors alone or in combination with epigenetic modulators.

Epigenetic targets and PD-L1 involvement

To date, numerous epigenetic targets, such as HDACs, DNMTs, LSD1, bromodomains, or EZH2 have been linked with the immunomodulation of PD-L1 (Figure 2).

HDACs

HDACs are divided into classes I, IIa, IIb, III, and IV, and act mainly via the removal of acetyl groups from the *N*-acetyl lysine amino acid residues of histones,[38] modulating gene expression and regulating PD-L1 expression.[39] Numerous HDAC inhibitors (HDACis) have been developed and are well reviewed elsewhere.[40] Apart from a direct cytotoxicity in cancer, HDACis were demonstrated to change immunogenicity and to enhance antitumoral immune responses by decreasing myeloid-derived suppressor cells (MDSCs) while simultaneously increasing the expression of the co-stimulatory molecules MHC-I/II, CD40, CD80, and CD86.[41,42]

Numerous HDACis, such as panobinostat (pan-HDACi), entinostat (class I HDACi), mocetinostat (HDAC 1,2,3 and 11 inhibitor) and tucidinostat, the first approved (in China) oral class I-selective HDACi, have been shown to increase the expression of PD-L1, mainly in melanoma models,[40,43] while showing a preference for class I selective agents. Most clinical data relating to the successful combination of PD-L1 inhibition and an HDACi relate to the pairing of entinostat and pembrolizumab (NCT02697630), resulting in a durable response in metastatic uveal melanoma.[44] Other combinations of entinostat with the bifunctional (TGF-β/PD-L1) fusion protein M7824 (NCT04708470), avelumab (NCT02915523), or nivolumab (NCT03838042) remain under evaluation.

In a clinical setting, Munster *et al.* investigated a triple combination of tamoxifen with the pan-HDACis vorinostat and pembrolizumab for the treatment of hormone-resistant breast cancer, with promising outcomes in some patients (NCT02395627),[45] whereas, for another triple therapy approach with entinostat, nivolumab, and ipilimumab (NCT02453620), no data are yet available. Promising first data are available for a Phase Ib study with vorinostat and pembrolizumab in patients with metastatic prostate carcinoma (NCT02619253).[46] There have been several studies colorectal cancer (CRC), but the results have yet to be reported.[37,47]

The selective inhibition of HDAC3 with RGFP966 increased the expression of PD-L1 by increasing histone acetylation on the PD-L1 promoter, which is the probable cause of resistance to RFGP966. Indeed, subsequent co-treatment with the anti-PD-L1 antibody BE101 enhanced the efficacy of RGFP966 in B cell lymphoma cells as well as in a mouse model.[48] By contrast, in pancreatic cancer cells, the same HDAC3i inhibitor reduced PD-L1 expression by modulating signal transducer and activator of transcription 3 (STAT3), indicating that HDAC3 inhibition could enhance immunotherapy.[49]

Recently, it was demonstrated that HDAC3 overexpression via the constitutive photomorphogenic 1 (COP1)/c-Jun axis inhibited PD-L1 expression in cisplatin-resistant NSCLC cells.[50] Taken together, HDAC3 appears to be able to up or downregulate histone acetylation of the PD-L1 promoter region; thus, further studies are necessary to determine whether selective HDAC3i is a suitable strategy for multidrug immunotherapy.

Similarly, HDAC6 can cause PD-L1 overexpression by modulating the STAT3 pathway, and HDAC6i with tubastatin A and nexturastat A impaired tumor progression in a melanoma mouse model.[51] A subsequent study by another research team provided insights into the mechanism of nexturastat A, which is able to increase the efficacy of anti-PD-L1 antibodies (CD279 and BE0146) by activation of immune surveillance cells and tumor-associated macrophages (TAMS), and not directly over T cells.[52] MPT0G612, another selective inhibitor of HDAC6, blocked IFNγ-mediated upregulation of PD-L1 in colorectal carcinoma cells, resulting in apoptosis via autophagy inhibition.[53]

Despite some available clinical data, our understanding of the effects of the combination of HDACis with immunotherapy, especially anti-PD-L1 therapy, remains limited. Further studies are necessary to understand better the complex biology behind these effects, as well as to develop better treatment options. Nevertheless, targeting HDACs appears to be a promising and effective approach to improving the response of the immune system to cancer cells.

DNMTs

DNMTs are important epigenetic enzymes, proven to be involved in the onset and development of numerous cancers.[54] To date, various DNMT inhibitors (DNMTis) have either been approved or are under clinical development. Interestingly, these inhibitors can upregulate MHC class I and II in many cancers,[55] such as decitabine in lymphoma. Furthermore, treatment with DNMTis resulted in the expression of several cancer testis antigens, which are cancer-associated antigens directly expressed on these malignant cells, of skin, lung, or colon cancers.[11,56] Thus, DNA methylation is likely a key player in the regulation of their expression. DNMTs also appears to have a crucial role in the maturation and differentiation of DCs because hypomethylated DNA enhancer regions are related to binding domains for transcription factors known to influence the DC lineage.[57]

Patients with resistance to PD-L1/PD-1 antibody treatment have low levels of activated immune cells; thus, activation of immune cells by DNMTis might boost the activity of PD-L1/PD-1 treatment. Indeed, combinations of DNMTis and anti-PD-L1 immunotherapeutic agents are under clinical investigation. Azacitidine is being tested in combination with durvalumab against metastatic $ER^+/HER2^-$ breast cancer (NCT02811497), whereas decitabine is being evaluated in co-treatment with pembrolizumab in locally advanced $HER2^-$ breast cancer (NCT02957968). The latter combination is also being tested on NSCLCs and esophageal carcinomas (NCT03233724).

Recently, the HDAC3i RFGP966 was reported to downregulate DNMT1 expression, resulting in enhanced PD-L1 levels.[48] The proposed mode of action of RFGP966 is a fascinating example of a complex epigenetically driven interplay of three targets: the cascade begins with direct HDAC3 inhibition and subsequent DNMT1 degradation, resulting in increased PD-L1 expression, which renders the tumor more susceptible to PD-1/PD-L1 blockade therapy. It is likely that HDAC3 inhibition does not directly influence DNA methylation of the PD-L1 promoter but instead triggers an IFN response via DNMT1 degradation, resulting in activated PD-L1 expression. However, further studies are necessary to confirm this mechanism.[58] Thus, it is of interest to study whether the acetylation levels of regulatory sequences for PD-L1 also correlate with their methylation.

However, the combination DNMT/PD-L1 inhibition is still in its infancy; thus, more studies are required to understand the potential of this novel combined therapeutic approach.

LSD1

LSD1, also known as KDMA1, is an essential epigenetic target mainly studied in the context of cancer. It is a histone demethylase able to modulate the methylation patterns of H3K4 and H3K9. Several inhibitors are currently in clinical trials for numerous forms of cancer, such as acute myeloid leukemia and solid cancers (e.g., breast or lung cancer).[59] Sheng *et al.* reported that PD-L1 inhibition, together with LSD1 ablation, might be a successful therapeutic strategy. Indeed, they were able to show that LSD1 depletion led to tumor immunogenicity and T cell infiltration in poorly immunogenic tumors, such as melanoma, restoring sensitivity to anti-PD-1 therapy. Despite these encouraging results, no pharmacological LSD1 inhibitor has received approval as yet.[60]

Moreover, LSD1 appears to have a crucial role in stemness maintenance and chemoresistance in breast cancer.[61] It is capable of transforming TAMs into M1-like macrophages, probably via the PD-L1 axis, in triple-negative breast cancer[62]; however, the involvement of PD-L1 remains rather elusive .

Interestingly, SP-2577, a novel reversible LSD1 inhibitor, enhanced antitumor immunity and T cell infiltration in an ovarian cancer model of the SWItch/Sucrose-NonFermentable (SWI/SNF) mutated complex. This compound activated IFN-dependent antitumor immunity and led to increased expression of PD-L1. A subsequent co-treatment with an anti-PD1 antibody resulted in a significantly enhanced immune response and decreased cancer cell growth.[63]

Besides the relevance in cancer, LSD1 has been demonstrated to be recruited to the PD-L1 gene locus via B lymphocyte-induced maturation protein 1 (Blmp-1) in viral infections. Thus, the removal of H3K4 methylation patterns via LSD1 inhibited expression of PD-L1.[64] Thus, a combination of LSD1 inhibitors together with an anti-PD-1 therapy is of high interest not only in regard of cancer, but also other immune-system-related diseases to increase therapy success and decrease resistance.

Bromodomains

Bromodomain and extraterminal (BET) motif inhibition is now considered a potential strategy in epigenetic-driven cancer therapy, but to date, no drugs have been approved despite intensive research.[65] BET proteins can control PD-1 expression in T cells as well as PD-L1 expression in cancer cell models. For example, BET inhibitors, such as JQ1, were shown to differentiate effector T cells into effector memory cells via suppression of the transcription factor BATF in a leukemia cell model.[66] PD-L1 is a target of JQ1 in neuroblastoma,[67] underlining the importance of the connection between PD-L1 and BET. In addition, in lung cancer, BET inhibition by JQ1 disrupted regulatory T cells (Tregs), and a concomitant PD-1 antibody treatment resulted in an improved antitumoral response in a mouse model.[68]

Moreover, promising results were recently published in triple-negative breast cancer,[69] highlighting targeting BET as a promising approach to overcome immune resistance and to improve antitumor therapy responses via reduction of the PD-1/PD-L1 axis. Based on these findings, several clinical trials are evaluating BET inhibitors other than JQ1 (i.e., SF1126, RO6870810, or BMS-986158) in combination with anti-PD-1 or anti-PD-L1 antibodies in patients with different cancers (NCT03059147, NCT03292172, and NCT02419417), further underlining PD-L1 inhibition with BET inhibitors as a valuable therapeutic approach.

EZH2

EZH2 isa well-known H3K27 histone methyltransferase, the overexpression of which is associated with numerous types of cancer. Various compounds have been studied as EZH2 inhibitors and, recently, tazemetostat entered the market.[70] Numerous clinical trials are ongoing, mainly limited to hematological malignancies.[71]

The role of EZH2 in immunology is not fully clear, because some studies suggest a positive influence on the immune response to fight cancer, whereas others suggest suppressed antitumor immunity. In 2019, two EZH2 inhibitors, MC4040 and MC4041, were

reported as being able to decrease proliferation in glioblastoma (GBM) cells and also to impair the GBM proinflammatory phenotype, with a significant decrease in TGFβ, TNFα, and IL6, with a concomitant increase in the anti-inflammatory cytokine IL10.[72]

Peng *et al.* reported that treatment with the EZH2 inhibitor GSK126 reactivated Th1-type chemokines, such as CXCL9 and CXCL10, thus allowing trafficking of effector T cells into the TME.[73] By contrast, EZH2 inhibition with GSK126 promoted the development of MDSCs, which are known to suppress an effective antitumor immune response.[74] EZH2 appears to have a crucial role in the differentiation of peripheral Tregs, which are suspected to influence the efficacy of PD-(L)1 immunotherapy.[75] Although EZH2 appears to be a double-edged sword in immunotherapy, the first clinical trial of a combination of the EZH2 inhibitor tazemetostat with pembrolizumab is underway in urothelial carcinoma.[76] However, more studies are necessary to understand the potential converging pathways of EZH2 and PD-L1 before proceeding with eventual co-treatment strategies.

PD-(L)1 small-molecule inhibition: a new frontier for cancer treatment?

As outlined above, PD-(L)1-specific antibodies have reached the market; however, despite remarkable positive outcomes in some patients, antibodies have well-known disadvantages when used as therapeutic agents, such as high costs, poor bioavailability, and above all, immune-related side effects[77]; thus, small molecules acting against the PD-1/PD-L1 pathway are urgently needed. Initially, researchers focused on small molecules able to target PD-(L)1, including peptides, peptidomimetics, or even macrocyclic peptides mainly developed by Aurigene or Bristol-Myers Squibb, often only described in patents with little or no research data available.[19] These early PD-(L)1 inhibitors are reviewed elsewhere.[17–19,22,78,79] Interestingly, despite the good validation of PD-(L)1 antibodies *in vitro* and *in vivo*,[22,80] the small molecules, even though very potent biochemically speaking, do not exhibit anticancer activity, or no data are available in this regard.[19,22,81]

Bristol-Myers Squibb, who pioneered the field of PD-(L)1 inhibition, also disclosed in 2015 the first nonpeptidic small molecules able to disrupt the PD-1/PD-L1 complex, exhibiting IC_{50} values of 1–300 nM[82,83]; BMS-202 and BMS-1018 with an *m*-(phenyloxymethyl)biphenyl scaffold have been prototypes for numerous subsequent molecules based on this pharmacophore,[22] which was described in detail showing the protein–protein interactions (PPIs) of the human PD-1/PD-L1 complex in detail.[83]

From these crystallographic studies, researchers understood that the binding surface of PD-(L)1 is relatively flat and hydrophobic, making the rational design of small molecules acting as PD-(L)1 inhibitors difficult; however, there is room for improvement because the numerous crystal structures of PD-(L)1 available alone or bound to various monoclonal antibodies[84] can provide inspiration for how to design and improve PD-L1 inhibitors by studying hot-spot residues in the aforementioned crystals in a dynamic environment. Figure 3 shows a schematic of the pharmacophore model as well as the key amino acids interacting with PD-L1 small molecules.

Most current PD-(L)1 modulators follow the precise pharmacophore model of compound 1: the biphenyl group appears to be vital and is the main driver of PD-L1 binding.[85] The ether linker does not appear to be crucial,[86] whereas a flexible central aromatic core connected, mainly in the *meta* or *para* position, to a polar tail appears to be essential for interactions with PD-L1. This polar tail often comprises an aminoethanol or a (substituted) ethylenediamine chain. Studies shed light on the mechanism of action of BMS-202 analogs because this molecule can lead to the formation of PD-L1 protein homodimers, hampering PD-L1 binding to its receptor PD-1 and, thus, blocking downstream signaling.[87,88] This mechanism is also likely to be valid for other related derivatives, reviewed elsewhere.[17–19,22,78,79] We highlight the most recent and/or promising small molecules in Table 1.

Concluding remarks and perspective

Chemotherapeutic agents have been used for decades as cancer therapy; however, the problems of relapse and resistance remain unsolved. The field of ICIs is rapidly evolving, and targeting PD-(L)1 has been proven to be particularly successful, as indicated by the FDA approval of several monoclonal antibodies[12]; however, the side-effects of antibody therapy are well known in terms of a low response because of intrinsic or acquired resistance, but also adverse immune effects limiting the use of these agents.[93] Safer treatment options are urgently needed, and research is focusing on peptidic and nonpeptidic small molecules acting on PD-(L)1.[94] Such molecules have numerous advantages over monoclonal antibodies, such as stability, membrane permeability, no immunogenicity, and last, but not least, oral administration. However, the development of these small-molecule immune checkpoint modulators is still in its infancy,[95] because no such inhibitor of the PD-(L)1 pathway has yet been approved, although the first promising examples have arrived in the clinic, such as CA-170, GS-4224, and INCB086550, with some promising data available.[23–25]

PPIs, such as PD-1/PD-L1, are challenging to target, as also seen for other PPIs, such as IL-2/IL-2R, p53/MDM2, BCL-2/BAX, GP120/CCR5, and Hsp90/Cdc37.[84] Few of these other targets have been successfully approached by small molecules, underlining the difficulties of the drug development process in this regard. As stated by Deng *et al.*, the discovery and development of most PD-L1 small molecules so far has often relied only on protein-based assays, such as homogeneous time-resolved fluorescence, without a clear translation into a more complex biological system, such as cells or mice; thus, their biochemical activity only might not be sufficient.[19] Previously, drug design has been based on a somehow static protein–ligand complex, but PPIs are highly dynamic and have to be studied as flexible plastic targets. The ample crystal structures of PD-(L)1 available, either alone or bound to various monoclonal antibodies were discussed in a recent perspective,[84] providing inspiration for how to design and improve PD-L1 inhibitors by studying hot-spot residues in the aforementioned crystals in a dynamic environment. For these approaches, advanced computational and biophysical techniques are required. These facts underline why small molecules against PD-L1 are hard to develop, even for big pharma.

Today, most small molecule PD-(L)1 modulators have a biphenyl ether-based moiety,[17,19,79] which appears to be vital for most of the highly active small molecules developed to date. Given that the crystal structures of numerous PD-L1 inhibitors are now available, it would be interesting to determine whether and how this moiety could be replaced to improve activity and pharmacokinetic/pharmacodynamic properties.[22]

Despite being nanomolar in biochemical assays, some PD-L1 inhibitors have not yet been validated in mice[22]; thus, they cannot be thoroughly evaluated. Therefore, the development of new structurally diverse PD-L1 inhibitors is of interest not only as improved

treatment options, but also as tools to understand better the complex biology behind them. Recently, compound 21 was described as a selective chemical probe[92] that will undoubtedly boost our understanding of the complex biology behind PD-L1 interactions.

Reprogramming of tumor cells via epigenetic mechanisms is crucial for escape from immune surveillance.[11] Over the past 30 years, epigenetic modulators have been under active development, resulting in valid therapeutic options[96]; however, the problems of relapse and resistance remain[12]; thus, urgent novel treatment options in the form of combination therapies are necessary.

As outlined above, the various steps in the immune cycle are regulated by epigenetic mechanisms, which can be influenced by epigenetic modulators. Some epidrugs have been demonstrated to improve antigen presentation, T cell trafficking, and migration, ultimately breaking the immunosuppressive state.[36]

As an immune checkpoint in combination with epigenetic therapeutic agents, PD-L1 is able to improve immune recognition and tumor growth, leading to increased response rates in preclinical and clinical studies.[36] Several combination approaches are currently under evaluation in numerous cancers, including colon, prostate, breast, and melanoma. However, these studies are mainly clinical, and the output is often unknown or not (yet) published. Combinations of epimodulators and antibodies against PD-L1 are still in their infancy and few results are available; nevertheless, this approach shows considerable therapeutic potential. However, we believe that research efforts also need to be directed toward evaluation of this combination approach by studying the crossroads of the potentially convergent molecular pathways so that combinations of PD-L1 antibodies and epigenetic modulators can be tested in a clinical setting, supported by sound scientific understanding.

Researchers are beginning to understand the interactions of PD-(L)1 with epigenetic enzymes. RFGP966 is a fascinating example of a complex interplay that results in direct HDAC3 inhibition, subsequent DNMT1 degradation, and increased PD-L1 expression, making the tumor more susceptible to PD-1/PD-L1 blockade therapy[48]; however, further studies are necessary to confirm the indirect mechanisms of action of this compound.[58] To sum up, although many questions remain, our increased understanding over the past few years underlines the fact that the combination therapy of epigenetic agents with immunomodulators is a rapidly evolving topic. In the years to come, we can expect a rapidly expanding knowledge and interest in the field of immune checkpoint therapy and epigenetic agents, from not only a biological, but also a therapeutic viewpoint.

Authors' contributions

All the authors contributed to the composition and revision of this article.

Acknowledgments

This work was supported by Italian Ministry of University FISR2019_00374 MeDyCa (A.M.). C.Z is thankful for the generous financial support of the FSE REACT-EU within the program PON 'Research and Innovation' 2014-2020, Action IV.6 'Contratti di ricerca su tematiche Green', as well as the funding from the KOHR GmbH, and the Sapienza Ateneo Project funding scheme.

Declaration of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1 Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018; 359(6382): 1350–1355.
2 Zou W, Chen L. Inhibitory B7-family molecules in the tumour microenvironment. *Nat Rev Immunol*. 2008; 8(6): 467–477.
3 Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol*. 2018; 18(3): 153–167.
4 Hui E, Cheung J, Zhu J, Su X, Taylor MJ, Wallweber HA, *et al.* T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science*. 2017; 355(6332): 1428–1433.
5 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(11): 3887–95.
6 Chen L. Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat Rev Immunol*. 2004; 4(5): 336–347.
7 Iwai Y, Hamanishi J, Chamoto K, Honjo T. Cancer immunotherapies targeting the PD-1 signaling pathway. *J Biomed Sci*. 2017; 24(1): 26.
8 Xie QK, He WZ, Hu WM, Yang L, Jiang C, Kong PF, *et al.* Tumor-infiltrating lymphocyte as a prognostic biomarker in stage IV colorectal cancer should take into account the metastatic status and operation modality. *Cancer Manag Res*. 2018; 10: 1365–1375.
9 Sharpe AH, Wherry EJ, Ahmed R, Freeman GJ. The function of programmed cell death 1 and its ligands in regulating autoimmunity and infection. *Nat Immunol*. 2007; 8(3): 239–245.
10 Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008; 26(1): 677–704.
11 Chen X, Pan X, Zhang W, Guo H, Cheng S, He Q, *et al.* Epigenetic strategies synergize with PD-L1/PD-1 targeted cancer immunotherapies to enhance antitumor responses. *Acta Pharm Sin B*. 2020; 10(5): 723–733.
12 Sanmamed MF, Chen L. A paradigm shift in cancer immunotherapy: from enhancement to normalization. *Cell*. 2019; 176(3): 677.
13 Brahmer J, Reckamp KL, Baas P, Crino L, Eberhardt WE, Poddubskaya E, *et al.* Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015; 373(2): 123–135.
14 Bellmunt J, de Wit R, Vaughn DJ, Fradet Y, Lee JL, Fong L, *et al.* Pembrolizumab as second-line therapy for advanced urothelial carcinoma. *N Engl J Med*. 2017; 376(11): 1015–1026.
15 Bottomley A, Coens C, Mierzynska J, Blank CU, Mandala M, Long GV, *et al.* Adjuvant pembrolizumab versus placebo in resected stage III melanoma (EORTC 1325-MG/KEYNOTE-054): health-related quality-of-life results from a double-blind, randomised, controlled, phase 3 trial. *Lancet Oncol*. 2021; 22(5): 655–664.
16 Boussiotis VA. Molecular and biochemical aspects of the PD-1 checkpoint pathway. *N Engl J Med*. 2016; 375(18): 1767–1778.
17 Surmiak E, Magiera-Mularz K, Musielak B, Muszak D, Kocik-Krol J, Kitel R, *et al.* PD-L1 inhibitors: different classes, activities, and mechanisms of action. *Int J Mol Sci*. 2021; 22: 11797.
18 Liu C, Seeram NP, Ma H. Small molecule inhibitors against PD-1/PD–L1 immune checkpoints and current methodologies for their development: a review. *Cancer Cell Int*. 2021; 21(1): 239.
19 Deng J, Cheng Z, Long J, Domling A, Tortorella M, Wang Y. Small molecule inhibitors of programmed cell death ligand 1 (PD-L1): a patent review (2019-2021). *Expert Opin Ther Pat*. 2022; 32(5): 575–589.
20 Zhang JY, Yan YY, Li JJ, Adhikari R, Fu LW. PD-1/PD-L1 based combinational cancer therapy: icing on the cake. *Front Pharmacol*. 2020; 11: 722.
21 Yang J, Hu L. Immunomodulators targeting the PD-1/PD-L1 protein-protein interaction: from antibodies to small molecules. *Med Res Rev*. 2019; 39(1): 265–301.
22 Pan C, Yang H, Lu Y, Hu S, Wu Y, He Q, *et al.* Recent advance of peptide-based molecules and nonpeptidic small-molecules modulating PD-1/PD-L1 protein-protein interaction or targeting PD–L1 protein degradation. *Eur J Med Chem*. 2021; 213: 113170.
23 Sasikumar PG, Sudarshan NS, Adurthi S, Ramachandra RK, Samiulla DS, Lakshminarasimhan A, *et al.* PD-1 derived CA-170 is an oral immune checkpoint inhibitor that exhibits preclinical antitumor efficacy. *Commun Biol*. 2021; 4(1): 699.
24 Zhang J, Zhang Y, Qu B, Yang H, Hu S, Dong X. If small molecules immunotherapy comes, can the prime be far behind? *Eur J Med Chem*. 2021; 218: 113356.
25 Fetse J, Zhao Z, Liu H, Mamani UF, Mustafa B, Adhikary P, *et al.* Discovery of cyclic peptide inhibitors targeting PD-L1 for cancer immunotherapy. *J Med Chem*. 2022; 65(18): 12002–12013.

26 Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. *N Engl J Med*. 2017; 377(25): 2500–2501.

27 Park YJ, Kuen DS, Chung Y. Future prospects of immune checkpoint blockade in cancer: from response prediction to overcoming resistance. *Exp Mol Med*. 2018; 50(8): 1–13.

28 Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature*. 2017; 541(7637): 321–330.

29 Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. *Science*. 2017; 357: eaal2380..

30 Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov*. 2006; 5(1): 37–50.

31 Baylin SB, Jones PA. Epigenetic determinants of cancer. *Cold Spring Harb Perspect Biol*. 2016; 8: a019505.

32 Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, *et al.* Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther*. 2019; 4: 62.

33 Wachowska M, Gabrysiak M, Golab J. Epigenetic remodeling combined with photodynamic therapy elicits anticancer immune responses. *Oncoimmunology*. 2014; 3: e28837.

34 Liu M, Zhou J, Chen Z, Cheng AS. Understanding the epigenetic regulation of tumours and their microenvironments: opportunities and problems for epigenetic therapy. *J Pathol*. 2017; 241(1): 10–24.

35 Ali MA, Matboli M, Tarek M, Reda M, Kamal KM, Nouh M, *et al.* Epigenetic regulation of immune checkpoints: another target for cancer immunotherapy? *Immunotherapy*. 2017; 9(1): 99–108.

36 Heninger E, Krueger TE, Lang JM. Augmenting antitumor immune responses with epigenetic modifying agents. *Front Immunol*. 2015; 6: 29.

37 Sedky NK, Hamdan AA, Emad S, Allam AL, Ali M, Tolba MF. Insights into the therapeutic potential of histone deacetylase inhibitor/immunotherapy combination regimens in solid tumors. *Clin Transl Oncol*. 2022; 24(7): 1262–1273.

38 Hesham HM, Lasheen DS, Abouzid KAM. Chimeric HDAC inhibitors: comprehensive review on the HDAC-based strategies developed to combat cancer. *Med Res Rev*. 2018; 38(6): 2058–2109.

39 Yi M, Niu M, Xu L, Luo S, Wu K. Regulation of PD-L1 expression in the tumor microenvironment. *J Hematol Oncol*. 2021; 14(1): 10.

40 Ho TCS, Chan AHY, Ganesan A. Thirty years of HDAC inhibitors: 2020 insight and hindsight. *J Med Chem*. Nov 12 2020; 63(21): 12460–12484.

41 Magner WJ, Kazim AL, Stewart C, Romano MA, Catalano G, Grande C, *et al.* Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. *J Immunol*. 2000; 165(12): 7017–7024.

42 Kim K, Skora AD, Li Z, Liu Q, Tam AJ, Blosser RL, *et al.* Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. *Proc Natl Acad Sci U S A*. 2014; 111(32): 11774–11779.

43 Bissonnette RP, Cesario RM, Goodenow B, Shojaei F, Gillings M. The epigenetic immunomodulator, HBI–8000, enhances the response and reverses resistance to checkpoint inhibitors. *BMC Cancer*. 2021; 21(1): 969.

44 Ny L, Jespersen H, Karlsson J, Alsen S, Filges S, All–Eriksson C, *et al.* The PEMDAC phase 2 study of pembrolizumab and entinostat in patients with metastatic uveal melanoma. *Nat Commun*. 2021; 12(1): 5155.

45 Munster PN, Terranova Barberio M, Pawlowska N, Moasser M, Chien AJ, Melisko ME, *et al.* Exhausted CD81 cells (Tex) to predict response to PD-1 therapy in estrogen receptor (1) hormone therapy resistant breast cancer predictive of response to immune checkpoint inhibitors after epigenetic priming. *Journal of Clinical Oncology* 2018; 36: 1044.

46 Pili R, Quinn DI, Albany C, Adra N, Logan TF, Greenspan A, *et al.* Immunomodulation by HDAC inhibition: results from a phase Ib study with vorinostat and pembrolizumab in metastatic urothelial, renal, and prostate carcinoma patients. *Journal of Clinical Oncology* 2019; 37: 2572.

47 Azad NS, Shirai K, McRee AJ, Opyrchal M, Johnson DB, Ordentlich P, *et al.* ENCORE 601: a phase 2 study of entinostat in combination with pembrolizumab in patients with microsatellite stable metastatic colorectal cancer. *Journal of Clinical Oncology*. 2018; 36(15_suppl): 3557.

48 Deng S, Hu Q, Zhang H, Yang F, Peng C, Huang C. HDAC3 inhibition upregulates PD-L1 expression in B-cell lymphomas and augments the efficacy of anti-PD-L1 therapy. *Mol Cancer Ther*. 2019; 18(5): 900–908.

49 Hu G, He N, Cai C, Cai F, Fan P, Zheng Z, *et al.* HDAC3 modulates cancer immunity via increasing PD-L1 expression in pancreatic cancer. *Pancreatology*. 2019; 19(2): 383–389.

50 Wang H, Fu C, Du J, Wang H, He R, Yin X, *et al.* Enhanced histone H3 acetylation of the PD-L1 promoter via the COP1/c-Jun/HDAC3 axis is required for PD-L1 expression in drug-resistant cancer cells. *J Exp Clin Cancer Res*. 2020; 39(1): 29.

51 Lienlaf M, Perez-Villarroel P, Knox T, Pabon M, Sahakian E, Powers J, *et al.* Essential role of HDAC6 in the regulation of PD-L1 in melanoma. *Mol Oncol*. 2016; 10(5): 735–750.

52 Knox T, Sahakian E, Banik D, Hadley M, Palmer E, Noonepalle S, *et al.* Selective HDAC6 inhibitors improve anti-PD-1 immune checkpoint blockade therapy by decreasing the anti-inflammatory phenotype of macrophages and down–regulation of immunosuppressive proteins in tumor cells. *Sci Rep*. 2019; 9(1): 6136.

53 Chen MC, Lin YC, Liao YH, Liou JP, Chen CH. MPT0G612, a novel HDAC6 inhibitor, induces apoptosis and suppresses IFN-gamma-induced programmed death-ligand 1 in human colorectal carcinoma cells. *Cancers (Basel)*. 2019; 11: 1617.

54 Conery AR, Rocnik JL, Trojer P. Small molecule targeting of chromatin writers in cancer. *Nat Chem Biol*. 2022; 18(2): 124–133.

55 Fonsatti E, Nicolay HJ, Sigalotti L, Calabro L, Pezzani L, Colizzi F, *et al.* Functional up-regulation of human leukocyte antigen class I antigens expression by 5-aza-2'-deoxycytidine in cutaneous melanoma: immunotherapeutic implications. *Clin Cancer Res*. 2007; 13(11): 3333–3338.

56 Palucka AK, Coussens LM. The basis of oncoimmunology. *Cell*. 2016; 164(6): 1233–1247.

57 Zhang X, Ulm A, Somineni HK, Oh S, Weirauch MT, Zhang HX, *et al.* DNA methylation dynamics during ex vivo differentiation and maturation of human dendritic cells. *Epigenetics Chromatin*. 2014; 7: 21.

58 Dai E, Zhu Z, Wahed S, Qu Z, Storkus WJ, Guo ZS. Epigenetic modulation of antitumor immunity for improved cancer immunotherapy. *Mol Cancer*. 2021; 20(1): 171.

59 Song Y, Zhang H, Yang X, Shi Y, Yu B. Annual review of lysine-specific demethylase 1 (LSD1/KDM1A) inhibitors in 2021. *Eur J Med Chem*. 2022; 228: 114042.

60 Sheng W, LaFleur MW, Nguyen TH, Chen S, Chakravarthy A, Conway JR, *et al.* LSD1 Ablation stimulates anti-tumor immunity and enables checkpoint blockade. *Cell*. 2018; 174(3): 549–563.

61 Verigos J, Karakaidos P, Kordias D, Papoudou-Bai A, Evangelou Z, Harissis HV, *et al.* The histone demethylase LSD1/kappaDM1A mediates chemoresistance in breast cancer via regulation of a stem cell program. *Cancers (Basel)*. 2019; 11: 1585..

62 Tan AHY, Tu W, McCuaig R, Hardy K, Donovan T, Tsimbalyuk S, *et al.* Lysine-specific histone demethylase 1A regulates macrophage polarization and checkpoint molecules in the tumor microenvironment of triple-negative breast cancer. *Front Immunol*. 2019; 10: 1351.

63 Soldi R, Ghosh Halder T, Weston A, Thode T, Drenner K, Lewis R, *et al.* The novel reversible LSD1 inhibitor SP-2577 promotes antitumor immunity in SWItch/Sucrose-NonFermentable (SWI/SNF) complex mutated ovarian cancer. *PLoS ONE*. 2020; 15(7): e0235705.

64 Bally APR, Neeld DK, Lu P, Majumder P, Tang Y, Barwick BG, *et al.* PD-1 expression during acute infection is repressed through an LSD1-Blimp-1 axis. *J Immunol*. 2020; 204(2): 449–458.

65 Mita MM, Mita AC. Bromodomain inhibitors a decade later: a promise unfulfilled? *Br J Cancer*. 2020; 123(12): 1713–1714.

66 Kagoya Y, Nakatsugawa M, Yamashita Y, Ochi T, Guo T, Anczurowski M, *et al.* BET bromodomain inhibition enhances T cell persistence and function in adoptive immunotherapy models. *J Clin Invest*. 2016; 126(9): 3479–3494.

67 Melaiu O, Mina M, Chierici M, Boldrini R, Jurman G, Romania P, *et al.* PD-L1 is a therapeutic target of the bromodomain inhibitor JQ1 and, combined with HLA Class I, a promising prognostic biomarker in neuroblastoma. *Clin Cancer Res*. 2017; 23(15): 4462–4472.

68 Adeegbe DO, Liu S, Hattersley MM, Bowden M, Zhou CW, Li S, *et al.* BET bromodomain inhibition cooperates with PD-1 blockade to facilitate antitumor response in Kras-mutant non-small cell lung cancer. *Cancer Immunol Res*. 2018; 6(10): 1234–1245.

69 Andrieu GP, Shafran JS, Smith CL, Belkina AC, Casey AN, Jafari N, *et al.* BET protein targeting suppresses the PD-1/PD-L1 pathway in triple-negative breast cancer and elicits antitumor immune response. *Cancer Lett*. 2019; 465: 45–58.

70 Tomassi S, Romanelli A, Zwergel C, Valente S, Mai A. Polycomb repressive complex 2 modulation through the development of EZH2-EED interaction inhibitors and EED binders. *J Med Chem*. 2021; 64(16): 11774–11797.

71 Italiano A, Soria JC, Toulmonde M, Michot JM, Lucchesi C, Varga A, *et al.* Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study. *Lancet Oncol*. 2018; 19(5): 649–659.

72 Stazi G, Taglieri L, Nicolai A, Romanelli A, Fioravanti R, Morrone S, *et al.* Dissecting the role of novel EZH2 inhibitors in primary glioblastoma cell cultures: effects on proliferation, epithelial-mesenchymal transition, migration, and on the pro-inflammatory phenotype. *Clin Epigenetics*. 2019; 11(1): 173.

73 Peng D, Kryczek I, Nagarsheth N, Zhao L, Wei S, Wang W, *et al.* Epigenetic silencing of TH1-type chemokines shapes tumour immunity and immunotherapy. *Nature*. 2015; 527(7577): 249–253.

74 Huang S, Wang Z, Zhou J, Huang J, Zhou L, Luo J, *et al.* EZH2 inhibitor GSK126 suppresses antitumor immunity by driving production of myeloid-derived suppressor cells. *Cancer Res*. 2019; 79(8): 2009–2020.

75 Bolton HA, Zhu E, Terry AM, Guy TV, Koh WP, Tan SY, *et al.* Selective Treg reconstitution during lymphopenia normalizes DC costimulation and prevents graft-versus-host disease. *J Clin Invest*. 2015; 125(9): 3627–3641.

76 Meeks JJ, Shilatifard A, Miller SD, Morgans AK, VanderWeele DJ, Kocherginsky M, *et al.* A pilot study of tazemetostat and MK-3475 (pembrolizumab) in advanced urothelial carcinoma (ETCTN 10183). *Journal of Clinical Oncology* 2020; 38: TPS607.

77 Wargo JA, Reuben A, Cooper ZA, Oh KS, Sullivan RJ. Immune effects of chemotherapy, radiation, and targeted therapy and opportunities for combination with immunotherapy. *Semin Oncol*. 2015; 42(4): 601–616.

78 Islam MK, Stanslas J. Peptide-based and small molecule PD-1 and PD-L1 pharmacological modulators in the treatment of cancer. *Pharmacol Ther*. 2021; 227: 107870.

79 Baglini E, Salerno S, Barresi E, Marzo T, Settimo FD, Taliani S. Cancer immunotherapy: an overview of small molecules as inhibitors of the immune checkpoint PD-1/PD-L1 (2015-2021). *Mini Rev Med Chem*. 2022; 22(14): 1816–1827.

80 Chen S, Song Z, Zhang A. Small-molecule immuno-oncology therapy: advances, challenges and new directions. *Curr Top Med Chem*. 2019; 19(3): 180–185.

81 OuYang Y, Gao J, Zhao L, Lu J, Zhong H, Tang H, *et al.* Design, synthesis, and evaluation of o-(biphenyl-3-ylmethoxy)nitrophenyl derivatives as PD-1/PD-L1 inhibitors with potent anticancer efficacy *in vivo*. *J Med Chem*. 2021; 64(11): 7646–7666.

82 Chupak LS, Zheng X. Bristol-Myers Squibb. Compounds useful as immunomodulators WO2015034820.

83 Chupak LS, Ding M, Martin SW, Zheng X, Hewawasam P, Connolly TP, *et al.* Bristol-Myers Squibb. Compounds useful as immunomodulators. WO2015160641.

83 Zak KM, Kitel R, Przetocka S, Golik P, Guzik K, Musielak B, *et al.* Structure of the complex of human programmed death 1, PD-1, and its ligand PD-L1. *Structure*. 2015; 23(12): 2341–2348.

84 Mittal L, Tonk R, Awasthi A, Asthana S. Traversing through the dynamic protein-protein interaction landscape and conformational plasticity of PD-1 for small-molecule discovery. *J Med Chem*. 2022; 65(8): 5941–5953.

85 Konieczny M, Musielak B, Kocik J, Skalniak L, Sala D, Czub M, *et al.* Di-bromo-based small-molecule inhibitors of the PD-1/PD-L1 immune checkpoint. *J Med Chem*. 2020; 63(19): 11271–11285.

86 Wang T, Cai S, Wang M, Zhang W, Zhang K, Chen D, *et al.* Novel biphenyl pyridines as potent small-molecule inhibitors targeting the programmed cell death-1/programmed cell death-ligand 1 interaction. *J Med Chem*. 2021; 64(11): 7390–7403.

87 Skalniak L, Zak KM, Guzik K, Magiera K, Musielak B, Pachota M, *et al.* Small-molecule inhibitors of PD-1/PD-L1 immune checkpoint alleviate the PD-L1-induced exhaustion of T-cells. *Oncotarget*. 2017; 8(42): 72167–72181.

88 Guzik K, Zak KM, Grudnik P, Magiera K, Musielak B, Torner R, *et al.* Small-molecule inhibitors of the programmed cell death-1/programmed death-ligand 1 (PD-1/PD-L1) interaction via transiently induced protein states and dimerization of PD-L1. *J Med Chem*. 2017; 60(13): 5857–5867.

89 Cheng B, Ren Y, Niu X, Wang W, Wang S, Tu Y, *et al.* Discovery of novel resorcinol dibenzyl ethers targeting the programmed cell death-1/programmed cell death-ligand 1 interaction as potential anticancer agents. *J Med Chem*. 2020; 63(15): 8338–8358.

90 Cheng B, Wang W, Niu X, Ren Y, Liu T, Cao H, *et al.* Discovery of novel and highly potent resorcinol dibenzyl ether-based PD-1/PD-L1 inhibitors with improved drug-like and pharmacokinetic properties for cancer treatment. *J Med Chem*. 2020; 63(24): 15946–15959.

91 Guo J, Luo L, Wang Z, Hu N, Wang W, Xie F, *et al.* Design, synthesis, and biological evaluation of linear aliphatic amine-linked triaryl derivatives as potent small-molecule inhibitors of the programmed cell death-1/programmed cell death-ligand 1 interaction with promising antitumor effects *in vivo*. *J Med Chem*. 2020; 63(22): 13825–13850.

92 Russomanno P, Assoni G, Amato J, D'Amore VM, Scaglia R, Brancaccio D, *et al.* Interfering with the tumor-immune interface: making way for triazine-based small molecules as novel PD-L1 inhibitors. *J Med Chem*. 2021; 64(21): 16020–16045.

93 Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med*. 2018; 378(2): 158–168.

94 Sasikumar PG, Ramachandra M. Small-molecule immune checkpoint inhibitors targeting PD-1/PD-L1 and other emerging checkpoint pathways. *BioDrugs*. 2018; 32(5): 481–497.

95 Wang T, Cai S, Cheng Y, Zhang W, Wang M, Sun H, *et al.* Discovery of small-molecule inhibitors of the PD-1/PD-L1 axis that promote PD-L1 internalization and degradation. *J Med Chem*. 2022; 65(5): 3879–3893.

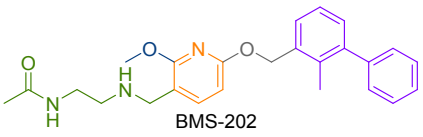
96 Tomaselli D, Lucidi A, Rotili D, Mai A. Epigenetic polypharmacology: a new frontier for epi-drug discovery. *Med Res Rev*. 2020; 40(1): 190–244.

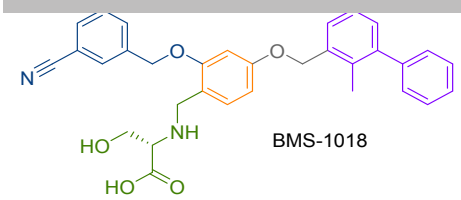
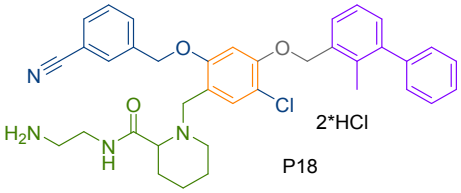
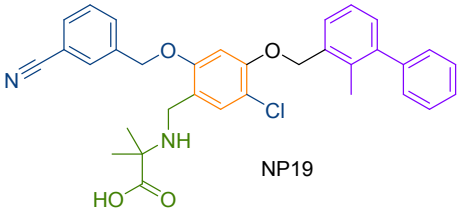
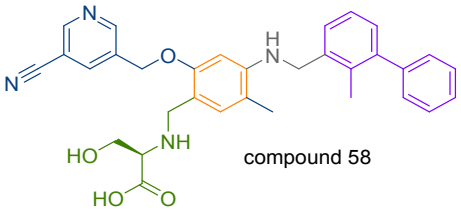
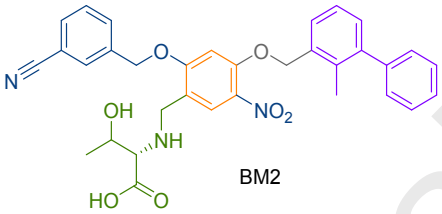
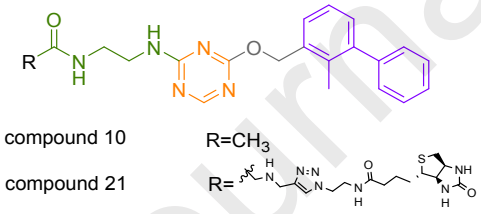
Figure 1. Chemical structures and data availability of programmed death-ligand 1 (PD-L1) inhibitors CA-170, GS-4224, and INCB086550 in clinical trials.

Figure 2. Epigenetic modulators in combination with programmed death-ligand 1 (PD-L1) antibodies: histone deacetylases (HDACs), DNA methyltransferases (DNMTs), lysine-specific demethylase 1 (LSD1), bromodomains, or enhancer of zeste homolog 2 (EZH2) small-molecule inhibitors currently used in (pre)clinical settings with various PD-(L)1 antibodies. Abbreviation: NSCLC, nonsmall cell lung cancer.

Figure 3. Programmed death-ligand 1 (PD-L1) small-molecule inhibitor pharmacophore model with a schematic of the main interactions with the amino acids of the binding site. Amino acids from chain A are depicted in red, whereas those of chain B are in blue.

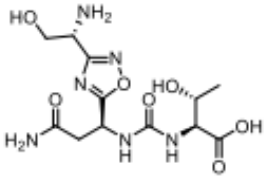
Table 1. Summary of the most promising recent PD-L1 small-molecule modulators

PD-L1 inhibitor	IC_{50}	Biological activity	Refs
 BMS-202	18 nM	Parent compound for most PD-L1 inhibitors; studied in numerous cellular and mouse models with various success, no transformation to the clinic	17–19,22,78,79

 BMS-1018	~10–30 nM	Similar to BMS-1018	17–19,22,78,79
 P18	9.1 nM	Active B16-F10 melanoma mouse model	89,90
 NP19	12.9 nM	Active in B16-F10 melanoma mouse model; positively evaluated in H22 model of liver cancer	89,90
 compound 58	12.0 nM	Active in humanized MC38 colon cancer mouse model, with similar potency to BMS-202 but with less unspecific toxicity	91
 BM2	2.7 nM	Active in Lewis lung carcinoma *in vitro* and in allograft mouse model, being more potent than BMS-1018	81
 compound 10 R=CH_3 compound 21 R=	115 nM for 10 and ND for 21	Compound 10 induces apoptosis more strongly than BMS-202 in PC9 or HCC827 lung cancer, despite being biochemically less potent. Compound 21 is first biotinylated probe available to further study its binding capability directly to PD-L1 on cell membranes	92

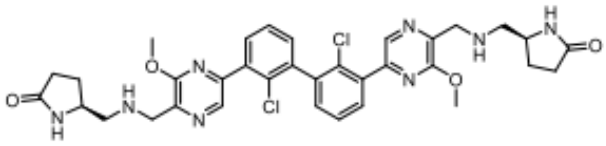
Highlights

- Short digest on the latest updates regarding immunotherapy with a focus on PD-L1
- Summary of the recent advances in the field of small molecule PD-L1 modulators
- Overview of the growing interest in rational therapy combinations of PD-L1 modulators with epi-drugs



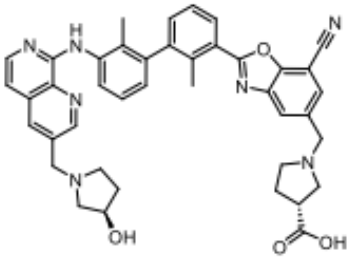
CA-170
IC_{50} 5-10 nM

I ?



GS-4224
IC_{50} not yet available

I ✓
II ?



INCB086550
IC_{50} <10 nM

I ✓
II ?

Key

I Clinical trial Phase I

II Clinical trial Phase II

✓ Results available

? Unknown/no results available yet

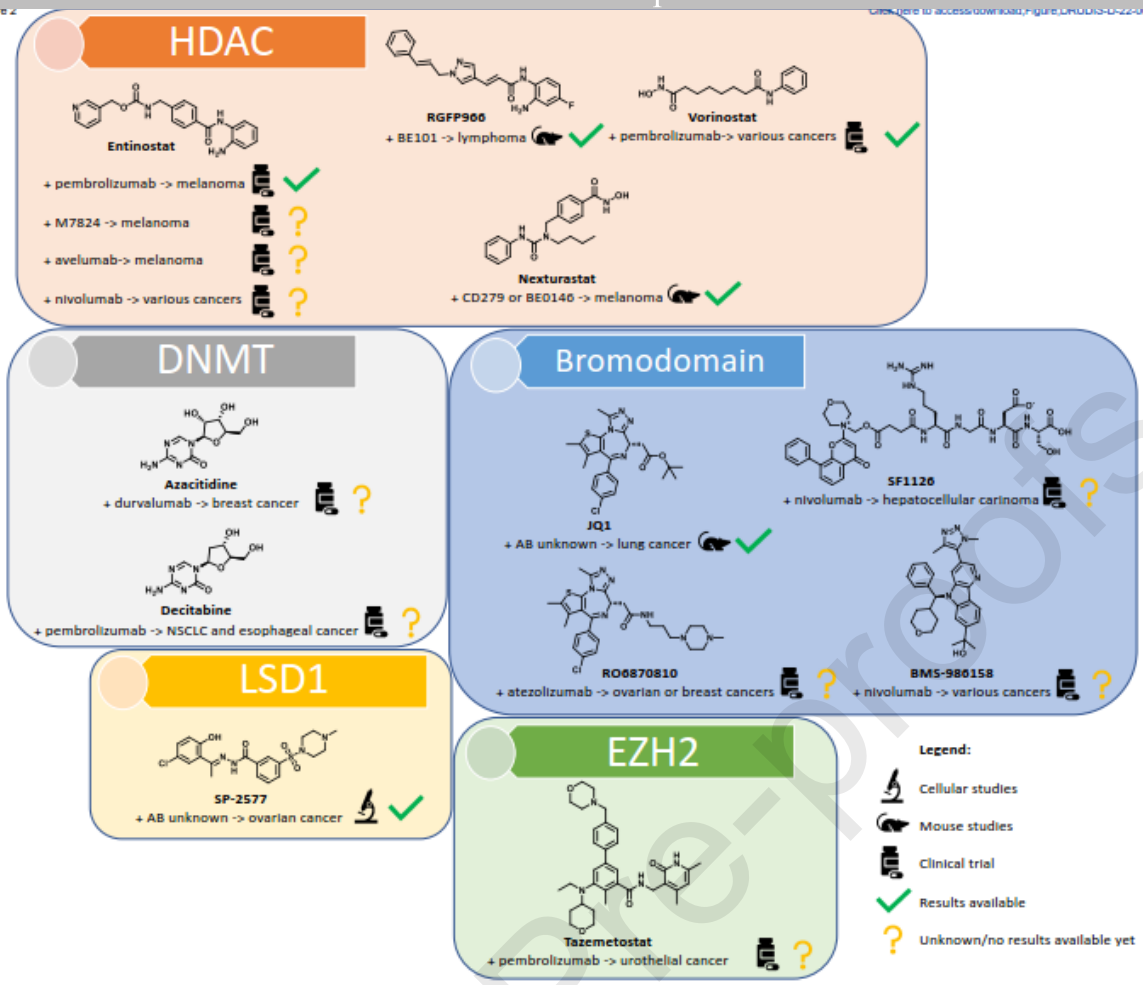


Figure 3