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Checkpoint Inhibition in Myeloma: Opportunities and Challenges

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Despite major improvements in the treatment landscape, most multiple myeloma (MM) patients eventually succumb to the underlying malignancy. Immunotherapy represents an attractive strategy to achieve durable remissions due to its specificity and capacity for long term memory. Activation of immune cells is controlled by a balance of agonistic and inhibitory signals via surface and intracellular receptors. Blockade of such inhibitory immune receptors (termed as “immune checkpoints”) including PD-1/PD-L1 has led to impressive tumor regressions in several cancers. Preclinical studies suggest that these immune checkpoints may also play a role in regulating tumor immunity in MM. Indeed, myeloma was among the first tumors wherein therapeutic efficacy of blockade of PD-1 axis was demonstrated in preclinical models. Expression of PD-L1 on tumor and immune cells also correlates with the risk of malignant transformation. However, early clinical studies of single agent PD-1 blockade have not led to meaningful tumor regressions. Immune modulatory drugs (IMiDs) are now the mainstay of most MM therapies. Interestingly, the mechanism of immune activation by IMiDs also involves release of inhibitory checkpoints, such as Ikaros-mediated suppression of IL-2. Combination of PD-1 targeted agents with IMiDs led to promising clinical activity, including objective responses in some patients refractory to IMiD therapy. However, some of these studies were transiently halted in 2017 due to concern for a possible safety signal with IMiD-PD1 combination. The capacity of the immune system to control MM has been further reinforced by recent success of adoptive cell therapies, such as T cells redirected by chimeric-antigen receptors (CAR-Ts). There remains an unmet need to better understand the immunologic effects of checkpoint blockade, delineate mechanisms of resistance to these therapies and identify optimal combination of agonistic signaling, checkpoint inhibitors as well as other therapies including CAR-Ts, to realize the potential of the immune system to control and prevent MM.

Keywords: myeloma, immunotherapy, immune checkpoint, PD-1, PD-L1

IMMUNE SYSTEM AS AN EFFECTIVE APPROACH TO TREAT CANCER AND PRINCIPLES OF IMMUNE CHECKPOINT BLOCKADE

The role of immune system in cancer progression has been studied for over a century (1). However, only recently immunotherapy has emerged as an effective strategy to treat several types of cancers with impressive results in terms of tumor regression and durable remissions (2). The concept of immune surveillance and editing of tumors is now well-accepted (3).

Several studies suggest a role for genetic and epigenetic modifications in cancer development and progression (4–6) and some of them correlate with the ability to escape this immunosurveillance (5, 6). Tumor cells can indeed lower their immunogenicity through the down regulation of MHC-mediated neo-antigen presentation, accompanied by deletion of cancer cells expressing T cell targets (immunoediting) (3). The immunoediting process in cancer pathogenesis comprises of three phases: elimination, equilibrium and escape. In the first phase, the innate and adaptive immune systems recognize and eradicate cancer cells through the cytolytic activity of immune cells (i.e., NK cells, NKT cells, $\gamma\delta$ T cells, and CD8⁺ T cells), antibody-dependent cell-mediated cytotoxicity (ADCC), or complement-dependent cytotoxicity (CDC) mechanisms (7, 8). In the equilibrium phase, a balance between cancer progression and cancer elimination is established through the modulation of control checkpoints (3, 7). However, if cancer persists, it overcomes the immunity response and escapes with further progression and metastasis (3, 7).

Along with the suppression of tumor antigen expression, different mechanisms that involve surface molecules and soluble factors released in the tumor microenvironment, e.g., indoleamine 2,3-dioxygenase (IDO), type I interferons (IFNs) and IFN- γ , galectin-1, have been described in the disruption of immune homeostasis and in the altered balance from effector to regulatory and suppressive cells induced by cancer (7, 9).

In principle, immunotherapy could either enhance the immune response or inhibit tumor suppression (10). The most commonly used approach is the modulation of inhibitory immune receptors (termed as “immune checkpoints”) that regulate the balance between immune response and immune tolerance (11). Several studies showed that cancer cells increase the expression of some checkpoint proteins (summarized in **Table 1**), such as programmed cell death ligand-1 (PD-L1), with inhibitory properties on T cell functions, as a mechanism of immune resistance (20). These results lead to the development of monoclonal antibodies (mAbs) directed against such immune checkpoints, further approved for the treatment of several solid tumors as melanoma, renal and lung cancer (21–23).

CTLA-4 is the first immune-checkpoint explored as a clinical target (24). It is normally expressed at low levels on the surface of effector T cells and regulatory T cells (Tregs) and it is involved in the early stages of T cell activation (25). CTLA-4 shares the same ligands of CD28 (CD80 and CD86) expressed on antigen presenting cells (APCs). Once CD28 binds CD80 or CD86 to provide co-stimulation, the inhibitory CTLA-4 molecule is shuttled to the T cell surface where it binds CD80 or CD86 with higher affinity (26) thus counteracting the costimulatory activity of CD28 through the binding of the phosphatases PP2A and SHP-2 (25, 27). CTLA-4 expression also exerts its immunosuppressive functions by other mechanisms, including Treg expansion and induction of immunosuppressive cytokines, such as transforming growth factor (TGF)- β and the enzyme IDO (13, 21). While CTLA4 expression is mostly studied for its expression on lymphoid cells, recent studies suggest that myeloid dendritic cells can secrete CTLA4⁺ microvesicles that may mediate immune suppression (28). CTLA-4 blockade with mAbs (i.e., ipilimumab)

can then enhance the immune response against tumor by inactivating Treg, tumor-infiltrating lymphocytes (TILs) (29) and increasing T helper (Th)1 cell functions (20).

PD-1 is a member of the CD28/CTLA-4 family, with inhibitory properties, mainly expressed on exhausted T cells (dysfunctional T cells classically associated with chronic infection), NK and NKT cells following activation (14). APCs, monocytes and malignant cells express its ligands, PD-L1 and PD-L2, especially under inflammatory conditions (14).

Similarly to CTLA-4, the interaction between PD-1 and PD-L1 interferes with TCR signal transduction, by recruiting the tyrosine phosphatase SHP-2 and subsequent inactivating the PI3 kinase-signaling cascade (30, 31), which leads to reduced cytokine synthesis, cytotoxic functions and blockade of T cell proliferation and survival (14).

In the physiologic setting, this pathway enables the immunologic equilibrium after initial T cell response, preventing over-activation and the possible expansion of auto-reactive T cells (32). Studies on PD-L1^{-/-} murine models reported an accumulation of effector T cells along with an increased IFN- γ production by CD8⁺ T cells, suggesting an impaired apoptosis regulation in the absence of PD-L1 (33). Moreover, spontaneous accumulation of CD8⁺ T cells occurred in the liver even in the absence of “non-self” antigen exposure leading to the development of multiple autoimmune features (33). These data highlighted the importance of PD-L1 in controlling the responses of self-reactive T cells that have escaped into the periphery. In addition, PD-1/PD-L1 axis regulates the dynamic interplay between Tregs and T effector cells. In the presence of inflammatory milieu, PD-L1 expressed on both APC and naïve Tregs induces PD-1 expression on naïve T cells and promotes their differentiation toward a regulatory phenotype and function (induced Tregs) (34). On the other hand, a negative feedback loop occurs to downregulate Treg development and function. This effect is mediated by the increased PTEN expression via PD-1 signaling which in turn reduces PD-L1 expression on Tregs (35). Finally, PD-L1⁺ Tregs directly induce a tolerogenic phenotype in APCs that reduces the priming of T effector cells (34). All these results thus confirmed the critical role of the PD-1/PD-L1 pathway in the balance between T cell activation and tolerance.

According to the relevance of PD-1/PD-L1 axis in immune control, tumors seem to hijack this pathway to suppress and escape the activation of an immune response (36). High PD-L1 expression is associated with a poor prognosis in solid tumors, including lung, ovarian or colon cancer, thus supporting the impressive results that PD-1/PD-L1 blockade has led in several cancers (36).

In addition to CTLA-4 and PD-1, other proteins with inhibitory properties, as Lymphocyte-activation gene (LAG)-3, T cell immunoglobulin (TIM)-3, T-cell immunoreceptor with Ig and ITIM domains (TIGIT) are currently under active investigations as potential targets for mAbs (37). LAG-3 is expressed on activated conventional T cells, Tregs, B cells and plasmacytoid dendritic cells (pDCs) (38) and the interaction with its major ligand, Class II MHC, inhibits conventional T cell activity while enhancing the suppressive function of

TABLE 1 | Immune checkpoint distribution and functions.

Checkpoint	Expression	Function	References
CTLA4 (CD80/CD86)	Activated T cells and Tregs	Inhibition of CD28 co-stimulation and T cell activity; Enhancement of Treg functions; Induction of TGF- β and IDO	(12) (13)
PD-1 (PD-L1/PD-L2)	Activated T cells, NK cells, NKT cells, B cells, Monocytes, DCs, MDSCs	T cell exhaustion and apoptosis; inhibition of cytokine production; downregulation of NK and NKT cell activity	(14)
LAG3 (MHCII)	activated T cells, NK cells, B cells, pDCs	Effector T cell inhibition; Increased Treg activity	(15)
TIM3 (Galectin 9, HMGB1)	Exhausted T cells, NK cells, NKT cells, B cells, DCs, Macrophages	Th1 cell apoptosis; Reduced cytokine release; Induction of tolerogenic M2 phenotype	(16) (17)
TIGIT (CD155)	Exhausted cytotoxic T cells, NK cells	Effector T cell inhibition; Reduced NK cell cytotoxicity; Enhanced Treg activity	(18) (19)

DCs, dendritic cells; MDSCs, myeloid derived suppressor cells; NK, natural killer; NKT, NK-like T cells; Th, T helper; Tregs, T regulatory cells.

Tregs (39). LAG-3 blockade in addition to anti-PD-1 strategy showed an additive therapeutic activity in preclinical models of chronic infection and cancer, according to their role as markers of exhaustion (40, 41). TIM-3 is another exhaustion-associated inhibitory receptor that blunts T-cell-effector function and induce T cell apoptosis (17). Mouse models of colon adenocarcinoma, melanoma, and sarcoma demonstrated anti-tumor activity of TIM-3 blockade especially in combination with PD-L1 blockade (42, 43). Moreover, anti-TIM-3 treatment increases the proliferation and cytokine production of CD8⁺ T cells derived from patients with melanoma (44). Anti-TIM-3 or anti-PD-L1 mAbs in combination with the blockade of TIGIT, a marker of exhausted cytotoxic cells, showed enhance anti-tumor activity in several animal models (45). Recent studies suggest promise for TIGIT blockade in future immunotherapy regimens without adding significant toxicity (19, 46).

In addition to checkpoint blockade, mAbs targeting agonist receptors, such as inducible co-stimulator (ICOS), OX40 and 4-1BB (47), are currently under clinical development, especially in solid tumors like melanoma (37).

The combined strategy to enhance T-cell activity with co-stimulatory mAbs and concurrently restoring T cell cytotoxic functions against cancer cells by blocking inhibitory proteins could be a promising approach (48). Several clinical trials on both solid and hematological malignancies are currently exploring this strategy (49).

REGULATION OF TUMOR IMMUNITY IN MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHIES OF UNDETERMINED SIGNIFICANCE

Multiple myeloma (MM) is a hematological malignancy characterized by clonal expansion of terminally differentiated B cells (plasma cells) in the bone marrow (BM). It is clinically manifested with osteolytic bone disease, infections, renal

insufficiency, and BM failure (50). The cross talk between malignant plasma cells (PCs) and the BM microenvironment, including immune cells, bone cells, endothelial cells, mesenchymal stromal cells (MSCs) and extracellular matrix, plays a pivotal role in the proliferation and survival of tumor cells (51).

Of note, “immunoparesis,” with a reduction in “uninvolved Igs,” is a common feature of MM (52). PC interactions with BM niche cells create a permissive microenvironment that can promote tumor growth and immune escape, through the production of several factors including TGF- β , interleukin (IL)-10, IL-6, and prostaglandin E2, known to have immunosuppressive properties (53). Among immune cells, DCs display an impaired differentiation and maturation in MM patients (54, 55) and their interaction with PCs enhance MM clonogenicity and proliferation through B cell activating factor (BAFF)/a proliferation inducing ligand (APRIL) signals (56, 57). Malignant PCs can in turn prompt DC fusion and trans-differentiation into osteoclasts (OCs) through receptor activator of nuclear factor κ B ligand and CD47 pathways (58–60), thus promoting immunosuppression and disease progression. Beside their role in bone remodeling, OCs also show immunosuppressive properties specifically inducing T-cell apoptosis through the up-regulation of immune checkpoint proteins as TIM-3 and the production of IDO and APRIL (61). These factors increase PD-L1 expression in MM cells thus supporting tumor escape from the immune control (61). DCs can also indirectly enhance osteoclastogenesis by promoting the expansion of T helper 17 (Th-17) clones in MM microenvironment (62) and the consequent accumulation of IL-17, known to be a potent pro-osteoclastogenic factor, in MM BM (60). Sponaas AM et al. reported that myeloid DCs also express PD-L1 and correlate with PD-L1⁺ PCs, suggesting that both cell types could contribute to the suppression of the anti-tumor T cell response in MM through PD-1/PD-L1 pathway (63). Furthermore, MM DC differentiation and maturation is inhibited by MSC production of immunosuppressive factors as IDO, IL-6, PTGS2

(64, 65). MSCs also increase PD-L1 expression on MM cells (66) which in turn suppress PD-1⁺ T cell and NK cell activity (67).

Along with PD-1/PD-L1 axis, a role for other inhibitory pathways, such as CD226 (68), and the induction of T-cell senescence (69) has also been implicated in the suppression of tumor immunity which characterized MM (68, 69). Several studies also reported an accumulation of myeloid derived suppressor cells (MDSCs) and Tregs, along with an unbalanced ratio of Th1/Th2 cells and dysfunctional NK cell cytotoxic activity in MM, compared to patients with monoclonal gammopathy of undetermined significance (MGUS) (70–72). Of note, this loss of function in several immune effector cells is associated with progression to clinical MM (73) and is in part due to the increased expression of suppressive factors, such as ligands of the activating receptor NKG2D (i.e., MHC class I chain-related protein A) from MGUS to MM (74).

More than 10 years ago it was demonstrated that the immune system can detect MGUS pre-neoplastic lesions and potentially control tumor growth (75). Indeed, the presence of CD4⁺ and CD8⁺ T cells, functionally active against pre-neoplastic cells and able to recognize a pattern of specific antigens for each patient tumor, was reported in the BM of MGUS patients (73). A further study identified SOX2 embryonal stem cell antigen as a distinct target of immunity in MGUS compared to MM (76). Interestingly, the presence of SOX2-specific T cells and PD-L1 expression on tumor cells and T cells at baseline was then found to be correlated with the risk of progression to MM (77). Of note, T cells against SOX2 were recently found to be implicated in durable response of a MM patient following chimeric-antigen receptor T (CART) cells (78).

Beside these mechanisms, the establishment of a chronic inflammatory status has been described in the evolution of asymptomatic diseases to MM (79), according to the tight correlation between inflammation and cancer development dating back to Virchow's studies in 1863. It is known that BM serum of MM patients is enriched of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12, IL-15, IL-17, IL-18, IL-22, IL-23, TNF- α , and IFN- γ (80). Moreover, a recent study from Botta C et al. interestingly defined an 8-genes signature (IL8, IL10, IL17A, CCL3, CCL5, VEGFA, EBI3, and NOS2) able to identify MGUS/smoldering/symptomatic-MM with 84% accuracy and built a prognostic risk score based on six genes (IFNG, IL2, LTA, CCL2, VEGFA, CCL3), validated in three additional independent datasets (79).

In the context of MM inflammatory status, bioactive lipids, typically increased during inflammation, may also play a crucial role in tumor development (81). In the past decade, obesity has indeed emerged as one of the risk factors for MM (82) and recent studies have shown an enrichment of lysophosphatidylcholine (LPC) species in MM patient serum compared to healthy donors (HDs) along with an expansion of CD1d-restricted type II NKT cell subsets, reactive against these lipids (83). These cells secrete high amounts of the immunosuppressive IL-13, thus supporting their role in the progression of the disease (83). On the other hand, a decline

as well as dysfunctional activation of type I NKT cells was also reported in MM patients, suggesting the balance between these two cell subsets as a new important immune-regulatory axis in the evolution of myeloma (83, 84). In support of this evidence, another study described that CD1d is highly expressed in premalignant and early MM and its expression decreases with disease progression (85). Dysregulation of lipid-reactive immune cells and a higher number of type II NKT cells, with enhanced capacity to promote PC differentiation, may be involved in the increased risk of gammopathy in Gaucher Disease, a lipid disorder (86–88). The multiplicity of mechanisms behind MM immunosuppression and enhancement of disease progression thus suggests the need of combinatorial approaches in the treatment of MM.

PRECLINICAL STUDIES TARGETING IMMUNE CHECKPOINTS IN MM

The role of PD-1/PD-L1 pathway in mediating immune escape of malignant PCs and the therapeutic efficacy of PD-1/PD-L1 blockade in other hematological malignancies led to an increased interest in the use of anti-PD-1/PD-L1 therapeutic strategies in MM (68). PD-L1 is highly expressed on PCs isolated from patients with MM, but not on normal PCs (66, 89–91). High PD-L1 expression on PCs was associated with disease progression in patients with MGUS and asymptomatic MM (77) and it could play a role in the development of clonal resistance as demonstrated by PD-L1 high levels in relapsed or refractory MM patients (66). Furthermore, PD-L1 upregulation emerged in patients with minimal residual disease, suggesting that residual PD-L1⁺ myeloma cells have an increased ability to survive and escape immunosurveillance (90). Nevertheless, high variability of PD-1/PD-L1 expression on PCs and BM niche cells was highlighted among patients with the same stage of disease (63, 90).

In vitro studies showed that MM microenvironment could induce PD-L1 expression on PCs; PD-L1 up-regulation indeed occurs in the presence of stromal cells (66) and PD-L1 blockade inhibits stromal cell-mediated PC growth (67). This effect is IL-6 dependent and mediated by STAT3, MEK1/2, and JAK2 pathways (66).

IFN- γ produced by cytotoxic T lymphocytes (CTLs) and NK cells strongly induces PD-L1 expression through the activation of MEK/ERK pathway (89). In addition, myeloid DCs, pDCs and MDSCs express PD-L1 in MM patients (63), with an increased proportion of PD-L1⁺ MDSCs in MM patients at remission compared to newly diagnosed and relapsed MM (92).

T cells from MM patients also display higher PD-1 expression levels, associated with loss of effector cell function (93) on both circulating T cells and BM CD8⁺ T and NK cells compared to HDs (67). Moreover, a study from Castella et al. (92) showed that PD-1 expression is already present on the anergic BM V γ 9V δ 2 T cell subset from MGUS patients and remained upregulated in MM after clinical remission (92). In contrast, PD-1 expression is reduced in T cells from patients who achieved minimal disease state following high dose chemotherapy (94).

In vitro studies further demonstrated that PD-1/PD-L1 blockade directly enhances NK and T cell mediated anti-MM responses (67, 93) and restores the capacity of PD-L1⁺ pDCs to induce cytotoxic activity of T cells and NK cells against MM PCs (95).

The effects of anti-PD-L1 mAb were also tested *in vivo*, on the 5T33 murine MM models, after autologous (syngeneic) stem-cell transplantation plus administration of a cell-based vaccine (96) or after irradiation (97). It was demonstrated that mice with advanced MM expressed higher levels of PD-1 on both CD8⁺ and CD4⁺ T cells compared to non-tumor bearing mice and the percentages of PD-1⁺ T cells correlated with the amount of tumor burden (97). Moreover, PD-1⁺ CD8⁺ T cells isolated from these mice showed a defective production of pro-inflammatory cytokines (IFN- γ and IL-2) after *in vitro* stimulation and expressed increased levels of the exhausted T cell marker TIM-3 (97). PD-1 blockade also prolonged the survival in disseminated myeloma-bearing mice (90, 96, 97) and this effect was abrogated by the depletion of CD4⁺ or CD8⁺ T cells, thus indicating the main role of both T cell subsets behind this strategy (96). Taken together, these studies supported the potential contribution of PD-1/PD-L1 pathway in the immune escape in MM and suggested that its blockade may be an effective therapeutic strategy against this tumor.

However, current evidences indicate that PD-1 blockade as single agent does not induce clinically meaningful anti-myeloma responses (98). In this regard, it was recently reported that the compromised functions of effector cells in MM may be due to senescence rather than PD-1 mediated exhaustion (69, 98). Exhausted T cells overexpress multiple inhibitory molecules, such as PD-1, CTLA-4, CD160, TIM-3 and LAG-3 and lack of IFN- γ expression (99). However, a PD-1^{low} T cell clonal expansion was observed in 75% of myeloma patients, in contrast to the non-clonal PD-1^{high} T cells (69, 98). This expanded population potentially represented tumor-reactive cells with a senescent phenotype. They indeed showed low levels of LAG-3, TIM-3, PD-1, and CTLA-4 and did not express CD27 and CD28, suggesting a late differentiated phenotype. Moreover, this clone expressed the typical senescent markers CD57, CD160 and KLRG-1 and displayed a secretory profile (69). In addition, it was described that the senescent phenotype was telomere independent as demonstrated by the low levels of p38-mitogen-activated protein kinase, p16 and p21 signaling pathways and it could be potentially reversed by other agents, as immunomodulatory drugs (IMiDs) or histone deacetylase inhibitors (69).

IMMUNOLOGIC EFFECTS OF IMiDS- RELEASING THE IKAROS CHECKPOINT

The development of the IMiDs, thalidomide (Thal) and its analogs lenalidomide (Len) and pomalidomide (Pom), has led to a paradigm shift in the treatment of MM (100). IMiDs exert their immunological functions through several mechanisms, including proliferation and functional enhancement of NK/NKT cells, induction of T-cell co-stimulation and reduction of Treg activity,

increased Th1 cytokine production, such as IL-2 and IFN- γ , anti-MM ADCC improvement and enhanced DC maturation and functions (101–103). The main molecular mechanism was recently elucidated showing that IMiDs bind Cereblon, causing a subsequent degradation of the transcriptional factors, Ikaros (IKZF1) and Aiolos (IKZF3) on both MM cells and T cells (104). Aiolos is a known repressor of the IL-2 gene promoter while Ikaros is also involved in the regulation of transcriptional silencing during Th2 differentiation (104–106).

Beside these effects, *in vitro* studies interestingly showed that Len treatment downregulates PD-1 expression on both T cells (93) and NK cells (67), restoring their cytotoxic activity, and decreases PD-L1 expression on malignant PCs and MDSCs (93). These data suggested that Len could enhance the effect of anti PD-1/PD-L1 blockade as further reported by Görgün G et al. *In vitro* studies (67).

Moreover, evaluation of immune function in MM patients treated with Pom demonstrated a poly-functional T-cell activation, with increased proportion of co-inhibitory receptor BTLA⁺ T cells and TIM-3⁺ NK cells (107), thus giving a rationale for the use of combination with immune checkpoint inhibitors. Analysis of the molecular mechanism of action revealed that Pom induces depletion of IKZF1 on both T and NK cells; however this effect is dependent on drug exposure and IKZF1 levels return back to baseline, prior to new cycle, with intermittent dosing (107). Interestingly, Pom-mediated immune activation correlated with clinical outcome even in heavily pretreated MM patients; although the baseline expression of Ikaros/Aiolos protein in tumor cells was not predictive of outcome (107).

More recently, a study from Bailur et al. (108) reported that Pom also reduces IKZF1 and IKZF3 levels on innate lymphoid cells (ILCs) and enhances their function, as demonstrated by the increased IFN- γ production both *in vitro* and *in vivo* (108). ILCs are a new subset of innate immune cells known to be involved in the regulation of immunity, inflammation and tissue homeostasis (109). The study also reported that ILCs are increased in BM of MGUS patients compared to HDs and their functions are enhanced in MGUS but decline in patients with asymptomatic MM (108). These results thus provided evidence that ILCs are among the earliest cell subsets enriched in the tumor microenvironment during the evolution of monoclonal gammopathies and represent a possible target to prevent disease progression by acting on their IKZF1 expression. In addition, PD-1 seems to be a negative regulator for ILC function (110) thus supporting the potential for synergy between IMiDs and anti-PD-1 mAbs in the treatment of MM.

EARLY CLINICAL STUDIES OF CHECKPOINT BLOCKADE AND COMBINATIONS IN MM

The preclinical evidence that PD-1/PD-L1 blockade enhances T cell and NK cell anti-MM cytotoxicity encouraged the use

of mAbs against these checkpoints in clinical trials. However, the use of anti PD-1/PD-L1 antibodies as monotherapy has not provided satisfying results. Specifically, a phase Ib clinical trial testing the anti-PD-1 Nivolumab (IgGk, fully human) in monotherapy reported no objective responses in 27 patients with relapsed or refractory MM (RRMM) (111). Similarly, a phase Ib trial of pembrolizumab (IgGk, humanized anti-PD-1) in monotherapy for RRMM (NCT01953692/KEYNOTE-013) described a stable disease in 57% of patients (112). Preliminary results of a phase II trial of pembrolizumab used in monotherapy as consolidation in MM patients (NCT02636010) demonstrated an increased depth of response in only 3 of 14 patients treated. This lack of efficacy could be explained by the low level of infiltrating effector cells that characterize MM, along with a relatively modest mutational burden as compared to solid tumors wherein therapeutic efficacy correlates with the mutational burden (113).

Lack of single agent activity led to studies testing PD-1/PD-L1 blockade as a part of a combined therapeutic strategy, particularly with IMiDs (**Table 2**). Pembrolizumab in combination with Len and dexamethasone (Dex) was evaluated in a phase I dose-escalation in 40 RRMM patients who experienced disease progression after more than two prior therapies (114). The objective response rate (ORR) in the whole population was 50%, with an ORR of 38% in Len-refractory patients (114). Preliminary results from the phase II clinical trial conducted on 48 RRMM patients, previously treated with a median of three regimens, showed an ORR of 60%, including 8% of stringent complete response/complete response, 19% VGPR, and 33% PR, with a median duration of response of 14.7 months (115, 116). Interestingly, a phase II study of Pembrolizumab following ASCT reported a CR rate of 31% at 6 months, including a 67% rate of BM MRD-negative state (117).

These results lead to the development of the phase III studies of pembrolizumab in combination with Len and Dex (KEYNOTE-185, NCT02579863) or Pom and Dex (KEYNOTE-183, NCT02576977) and one phase III study of Pom and Dex vs. nivolumab, Pom, and Dex vs. nivolumab, elotuzumab, Pom, and Dex (CheckMate 602, NCT02726581). However, in June 2017 the US Food and Drug Administration transiently halted the clinical trials of anti-PD-1/PD-L1 mAbs in combination with IMiDs, due to an imbalance of deaths in the Pembrolizumab arms in KEYNOTE-183 and KEYNOTE-185 and no significant differences in terms of objective response (<https://www.fda.gov/Drugs/DrugSafety/ucm574305.htm>). As these studies have not yet been published in a peer-reviewed format, more details that might shed light on the possible explanations for these observations are lacking. With further review of safety data on ongoing trials, some of the studies of combinations of PD-1/PD-L1 blockade in MM have now been reinitiated. Combinations of PD-1 blockade with other MM therapies are also currently under evaluation. Preliminary results on a phase I trial of the anti PD-1 Nivolumab in combination with other established anti-myeloma agents (Len/Pom, Dex, anti-CD38 Daratumumab, proteasome inhibitors) revealed acceptable safety profile in refractory, heavily pre-treated,

high-risk MM patients (118). In addition anti-PD-1 based therapy, clinical trials of mAbs targeting PD-L1 (Atezolizumab and Durvalumab), both alone and in combination with other agents (i.e., Elotuzumab, and Daratumumab) have also been developed.

Together, these studies point to the need for careful evaluation of immune checkpoint strategies and their combinations in MM, with cautious attention to toxicities as well as pharmacodynamics endpoints.

MAJOR UNMET NEEDS AND FUTURE DIRECTIONS

The concept that immune system can regulate the growth of MM cells is now well-established and immune-based approaches carry the promise of long term disease control and even cure without the need for ongoing therapy. Current MM therapies, such as IMiDs and anti-CD38 antibodies can have immunologic effects; newer therapies particularly CAR-T cells and T cell-engaging bi-specifics are in active clinical investigation and showing promising results. However, there remains an unmet need to address the mechanisms operative in the tumor microenvironment that restrict or prevent long term control of tumors.

Further studies are needed to better understand the mechanisms behind the lack of clinical activity of single agent PD-1 blockade in MM. Several mechanistic possibilities exist, including dominance of other inhibitory checkpoints, immune suppressive cells, lack of agonistic signaling, the low number of tumor-specific T cells in the tumor microenvironment, poor antigen presentation, low mutational burden of MM tumors, as well as senescence of tumor-infiltrating T cells. Moreover, MM is not a single disease and it consists of several distinct genetic subtypes; thus, it is likely that immune microenvironment in MM may also differ between patients. This heterogeneity may even be spatial within the same patient, as recently illustrated for solid tumors (120). As MM is a malignancy involving an immune cell, it is also theoretically possible that PD-1 blockade may lead to altered cross-talk with other immune cells and paradoxically promote tumor growth. It is also of interest to identify if there are specific subsets of patients (such as those with high mutational burden on MM cells), who might preferentially benefit from checkpoint blockade.

Some of the possibilities discussed above suggest the chance that the lack of efficacy of PD-1 blocking antibodies as single agents can be reverted by the combination with other agents. This strategy could lead to distinct pharmacodynamics effects as well as toxicity profiles compared to monotherapies (121).

It should be noted that many of the published data involve PD-1 targeted therapies; however, the effects of PD-L1 blockade may differ.

Preclinical studies also suggest a potential efficacy of agonistic antibodies in preclinical models as well. As an example, anti-CD137 Abs were shown to lead to strong tumor immunity in

TABLE 2 | Selected clinical trials of checkpoint inhibitor-based therapies in Multiple Myeloma.

Study	Phase	Clinical trial identifier	References
A study of pembrolizumab (mk-3475) in combination with standard of care treatments in participants with multiple myeloma (MK-3475-023/KEYNOTE-023)	I	NCT02036502	(114)
An investigational immuno-therapy study to determine the safety and effectiveness of nivolumab and daratumumab, with or without pomalidomide and dexamethasone, in patients with multiple myeloma	I	NCT01592370	(118)
Study of lenalidomide/dexamethasone with nivolumab and ipilimumab in patients with newly diagnosed multiple myeloma	I	NCT03283046	–
A study to determine dose and regimen of durvalumab as monotherapy or in combination with pomalidomide with or without dexamethasone in subjects with relapsed and refractory multiple myeloma	I	NCT02616640	–
A study of PVX-410, a cancer vaccine, and durvalumab ± lenalidomide for smoldering MM	I	NCT02886065	–
A study of atezolizumab (anti-programmed death-ligand 1 [PD-L1] antibody) alone or in combination with an immunomodulatory drug and/or daratumumab in participants with multiple myeloma (MM)	Ib	NCT02431208	–
A study of durvalumab in combination with lenalidomide with and without dexamethasone in subjects with newly diagnosed multiple myeloma	Ib	NCT02685826	–
1454GCC: Anti-PD-1 (MK-3475) and IMiD (Pomalidomide) combination immunotherapy in relapsed/refractory multiple myeloma	I/II	NCT02289222	(115, 116)
Pembrolizumab cyclophosphamide and lenalidomide for patients with relapsed multiple myeloma (MUKfourteen)	I/II	NCT03191981	–
Pembrolizumab, lenalidomide, and dexamethasone in treating patients with newly diagnosed multiple myeloma eligible for stem cell transplant	II	NCT02880228	–
Phase 2 multi-center study of anti-pd-1 during lymphopenic state after HDT/ASCT for multiple myeloma	II	NCT02331368	(117)
Pembrolizumab + Lenalidomide post-autologous stem cell transplant (ASCT) in high-risk multiple myeloma (MM)	II	NCT02906332	(119)
Efficacy and safety study of pembrolizumab (MK-3475) in combination with daratumumab in participants with relapsed refractory multiple myeloma (MK-3475-668/KEYNOTE-668)	II	KEYNOTE-668 NCT03221634	–
A study of elotuzumab in combination with pomalidomide and low dose dexamethasone and elotuzumab in combination with nivolumab in patients with multiple myeloma relapsed or refractory to prior treatment with lenalidomide	II	NCT02612779	–
An exploratory study to evaluate the combination of elotuzumab and nivolumab with and without pomalidomide in relapsed refractory multiple myeloma	II	NCT03227432	–
A Phase II trial if nivolumab, lenalidomide and dexamethasone in high risk smoldering myeloma	II	NCT02903381	Based on ClinicalTrials.gov. U.S. National Library of Medicine [https://clinicaltrials.gov/]. Accessed 2 Jan 2018.
A study to determine the safety and efficacy for the combination of durvalumab and daratumumab in relapsed and refractory multiple myeloma (FUSIONMM-003)	II	NCT02807454	–
A study to determine the efficacy of the combination of Daratumumab (DARA) plus Durvalumab (DURVA) (D2) in subjects with Relapsed and Refractory Multiple Myeloma (RRMM) (FUSION-MM-005)	II	NCT03000452	–
Study of pomalidomide and low dose dexamethasone with or without pembrolizumab (MK-3475) in Refractory or Relapsed and Refractory Multiple Myeloma (rrMM) (MK3475-183/KEYNOTE-183)	III	KEYNOTE-183/ NCT02576977	https://www.fda.gov/Drugs/DrugSafety/ucm574305.htm
Study of lenalidomide and dexamethasone with or without pembrolizumab (MK-3475) in participants with newly diagnosed treatment naive multiple myeloma (MK3475-185/KEYNOTE-185)	III	KEYNOTE-185/ NCT02579863	https://www.fda.gov/Drugs/DrugSafety/ucm574305.htm

VKappa-myc MM models (122, 123). Although a small study with this agent in MM was not completed, further evaluation of this pathway particularly in combination may be of interest. T cells in MM lesions also express other inhibitory molecules, such as TIM-3 and LAG-3. Antibodies targeting these molecules are now entering the clinic and the effects of these agents in human MM are awaited. In addition to their effects on T cells, immune regulatory pathways are also operative for innate cells, such as NK-T cells and ILCs. These pathways may also limit the efficacy of engineered T cells, such as CAR-T cells, as well as bispecifics. Future combinations of these strategies to harness immune-mediated MM control are therefore eagerly awaited.

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Conflict of Interest Statement: MVD has served on advisory boards for BMS, Merck and Genentech/Roche.

The remaining 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.

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