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## The Challenges of Checkpoint Inhibition in the Treatment of Multiple Myeloma

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### Abstract

Despite significant improvements in the overall survival of patients with multiple myeloma (MM) over the past 15 years, the disease remains incurable. Treatment options are limited for patients who have relapsed or are refractory to immunomodulatory drugs (IMiDs), proteasome inhibitors, and monoclonal antibodies. In these patients, immunotherapies such as checkpoint inhibitors, oncolytic vaccines, and chimeric antigen receptor (CAR) T cells provide a potentially effective alternative treatment. While checkpoint inhibitors are effective in prolonging overall survival in some patients with advanced solid cancers and Hodgkin lymphoma, they have not demonstrated significant anti-myeloma activities as a single agent in MM. In fact the combination of checkpoint inhibitors with IMiDs was recently found to increase the risk of death in myeloma patients. These challenges highlight the need for a better understanding of immune dysregulation in myeloma patients, and the mechanisms of action of- and resistance to- checkpoint inhibitors. In this review, we summarize immune dysfunction in patients with MM, and review the preclinical and clinical data regarding checkpoint inhibitors in myeloma. We conclude by proposing strategies to improve the efficacy and safety of checkpoint inhibitors in this population.

### Keywords

multiple myeloma; immunotherapy; checkpoint inhibitors; CTLA4; PD-1/PD-L1

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Authors' contributions

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Competing interests

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## I. Multiple myeloma

Multiple Myeloma (MM) is a malignancy that is characterized by the clonal proliferation of terminally differentiated plasma cells within the bone marrow. MM represents 1% of all malignancies and 18% of hematologic malignancies in the United States accounting for an estimated 30,770 new diagnoses and 12,770 deaths in 2018 alone<sup>1</sup>. Classically, MM results in the secretion of a non-functional monoclonal immunoglobulin (Ig) that is produced by the transformed plasma cells. Production of this aberrant Ig results in several of the complications associated with MM such as renal dysfunction, neuropathy, and hyperviscosity syndrome<sup>23</sup>. However, in approximately 15–20% of patients the abnormal plasma cells secrete only monoclonal free light chains, and in approximately 2–3% of cases these cells secrete no monoclonal protein at all, resulting in the so-called non-secretory myeloma<sup>45</sup>. Myeloma cell growth in the bone marrow and the resultant cytokines produced by these transformed cells lead to the classic symptoms of active MM: osteolytic bone lesions, hypercalcemia, and anemia<sup>6</sup>.

For decades, low dose melphalan and prednisone constituted the cornerstone of MM treatment. However, complete responses with this regimen are rare, and the median time to progression is less than 15 months<sup>7,8</sup>. A significant advance in the management of MM came from the upfront use of high doses of melphalan with autologous hematopoietic stem cell transplantation (AHSCT). This treatment has allowed for improved response rates, progression free survival, and—in some trials—prolonged survival in MM patients<sup>9–12</sup>. High dose melphalan with AHSCT continues to be a fundamental therapeutic modality in younger patients after response to conventional induction therapy and/or at the time of disease progression.

The subsequent advent of new “biologic” agents in MM treatment regimens has led to marked improvement in the depth and duration of the responses obtained. The immunomodulatory drugs (i.e., thalidomide, lenalidomide, and pomalidomide), along with proteasome inhibitors (i.e., bortezomib, carfilzomib, and ixazomib) have shown significant efficacy in the management of both newly diagnosed as well as relapsed and refractory MM patients<sup>13–16</sup>. Monoclonal antibodies such as daratumumab and elotuzumab were approved by the FDA in 2015 and provide additional options for the treatment of MM<sup>17–22</sup>. Treatment of MM patients with combination regimens that contain one or more biologic agents, followed by consolidation with AHSCT has resulted in the highest response rates yet in newly diagnosed MM patients<sup>23–28</sup>.

Despite the use of the “biologic” agents and the incorporation of AHSCT, MM remains an incurable disease, with some patients relapsing within months after autologous hematopoietic stem cell transplantation. Nearly all MM patients will eventually develop resistance to currently available agents; as such there is a significant unmet medical need for the development of novel therapeutic agents against myeloma. Immunotherapy—i.e., agents which stimulate a patient’s own immune system to attack cancer cells—has recently moved to the forefront of cancer treatment, and has yielded unprecedented tumor responses and long-term survival benefits in patients with advanced solid malignancies and Hodgkin lymphoma<sup>29–32</sup>. Immunotherapy utilizes one of four major strategies: targeting tumor

surface antigens with a monoclonal antibody; adoptive transfer of immune effector cells including chimeric antigen receptor T cells; boosting tumor-specific immunity using vaccines; and inhibiting tumor specific immune suppression with checkpoint inhibitors<sup>33</sup>. This review focuses on the role of checkpoint inhibitors that target the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and the programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) in the treatment of MM.

## II. Overview of the human immune system

The human immune system consists of two main components: the innate immune system, and the adaptive immune system. Innate immunity serves as the initial defense mechanism against microbial invasion and is characterized by a rapid, nonspecific response to pathogens. The components of the innate immune system include natural anatomic barriers (skin and mucosa), soluble proteins, bioactive small molecules (cytokines and complement components), and several types of myeloid derived leukocytes which typically express cell-surface toll-like receptors (TLRs). All elements of the innate immune system are encoded by the host's germline DNA. They are preformed and able to react to the first sign of pathogenic invasion.

Unlike the innate immune system, the adaptive immune response is encoded by genetic elements that undergo somatic rearrangement to form antigen-binding molecules with a unique specificity for individual antigens. The adaptive immune system is mainly derived from lymphoid progenitor cells and includes both cellular and humoral immunities. The effector cells of the adaptive immune system are B cells and T cells. The T-lymphocyte population can be further subdivided into CD4<sup>+</sup> (T helper cells), CD8<sup>+</sup> (cytotoxic T cells) and regulatory T cells (T<sub>regs</sub>). Upon exposure to antigens, the T and/or B effector cells undergo clonal expansion to mount an effective response. This adaptive response produces long-lived memory T and/or B cells. These memory cells will persist in a dormant state, but can undergo rapid expansion if they ever encounter the antigen again; resulting in a more rapid, robust immune responses upon subsequent exposures to a specific antigen. B cells mediate the humoral immune response by producing antibodies against specific antigens either alone or with the help of CD4<sup>+</sup> T cells. Cellular immunity is mainly mediated by CD8<sup>+</sup> T cells, which eliminate pathogens and tumor cells via apoptosis or cellular lysis through the release of perforins and granzymes.

Classically, full CD4<sup>+</sup> and CD8<sup>+</sup> T-cell activation requires two signals. Signal 1 is provided by the interaction between the T-cell receptor (TCR) and the peptide-MHC complex on the antigen-presenting cells (APCs). Signal 2 is a costimulatory signal mediated by the interaction between the costimulatory molecule CD28 on the T cell and CD80 (B7.1) or CD86 (B7.2) on the APC<sup>34,35</sup>. Both signal 1 and signal 2 are required for full T-cell activation. In the absence of the costimulatory signal, the interaction between the TCR and the peptide-MHC complex results in T-cell anergy<sup>36–38</sup>. Cytotoxic T lymphocyte antigen 4 (CTLA4) was subsequently identified as a coinhibitory molecule that can induce the downregulation of CD28 by endocytosis and compete with CD28 for binding with CD80/CD86. CTLA4 can thus dampen the T cell response and prevent aberrant and autoreactive immune responses<sup>39–42</sup>.

Many other cell surface signaling molecules have been identified over the last 20 years (for review, see ref<sup>43</sup>) and the two-signal model has evolved into an increasingly complex regulatory system, in which both co-stimulatory and co-inhibitory molecules have been discovered. Most of these co-signaling molecules belong to either the immunoglobulin superfamily (IgSF) or the tumor necrosis factor receptor superfamily (TNFRSF). Among the IgSF receptors, the CD28 family (CD28, ICOS, CTLA4, PD1, PD1H, and BTLA) and B7 family (B71, B7H1/PDL1) are the most well-described members. Additional IgSF cosignaling receptors—type I transmembrane immunoglobulin and mucin (TIM) domain-containing molecules, CD2/signaling lymphocytic activation molecule (SLAM) family members, Butyrophilin (BTN) and BTN-like (BTNL) family molecules, Lymphocyte activation gene 3 protein (LAG3), and CD226 family members (CD226, CRTAM, TIGIT, and CD96)—have also been reported<sup>44–47</sup>. The TNFRSF receptors contain one or more extracellular cysteine-rich domain and are divided into 4 major subfamilies: Type-V (4–1BB, OX40, CD27, GITR, CD30); Type-L (TNFR1, TNFR2, HVEM, TNFRSF3, TNFRSF25, TNFRSF6B, FAS, CD40, RANK, OPG, TRAILR1–4); Type-S (TACI, BAFFR, BCMA, TWEAKR, EDAR); and orphan.

Co-signaling molecules positively and negatively regulate T cell fate and function, and thus maintain the equilibrium between immune activation and tolerance. The level of expression of co-signaling molecules on T cells is highly variable and context dependent. In contrast to the “on/off” models of co-signaling, it was recently proposed that the repertoire of costimulatory and co-inhibitory receptors is varied continuously in response to dynamic environmental conditions: the so called “tidal model” of co-signaling<sup>43,48</sup>. This model postulates that at the onset of T-cell activation there is an abundance of co-stimulatory receptors on naïve and activated T cells, resulting in the functional responsiveness of these cells (the so-called “rising tide”). This is followed by expression of both co-stimulatory and co-inhibitory molecules; T cell functionality is ultimately directed by the relative strength of these two opposing actions (the so-called “peak tide”). Finally, the relative expression of co-inhibitory receptors increases leading to suppression of further T cell activation (the so-called “receding tide”).

CTLA4/CD28 and the programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathways are the most well characterized negative regulators of immune activation. Similar to other co-signaling molecules, CTLA4/CD28 and PD-1/PD-L1 are subject to spatiotemporal regulation and undergo bi-directional co-signaling. TCR signaling and co-signaling molecules form the immune synapse, which is composed of the central, peripheral, and distal supra-molecular activation complexes (SMAC) with functionally diverse co-signaling molecules. CD28, CD4/CD8 and the TCR form the central SMAC (cSMAC), which initiates the recruitment of signaling molecules and T cell activation<sup>49</sup>. Following activation, the co-inhibitory molecules CTLA4 and PD-1 are recruited to the immune synapse. CTLA4 and possibly PD-1 accumulate in the same region of the cSMAC as CD28 to physically exclude CD28 from the cSMAC<sup>50,51</sup>. CTLA4 induces the downregulation of CD28 by endocytosis<sup>39–42</sup>. CTLA4 and PD-1 recruit SH2 domain-containing tyrosine phosphatase 1 (SHP1), SHP2, and serine/threonine protein phosphatase 2A (PP2A), which suppress T cell activation by dephosphorylating several of the major signaling pathways that are essential for T cell activity<sup>51,52</sup>.

The interactions between co-signaling molecules and their counter-receptors (ligands) are bi-directional: the interacting molecules on each respective cell function to transduce a signal into that particular cell<sup>43</sup>. When CTLA4 on T cells binds to B7-1 and B7-2, it transmits signals into the T cell that inhibit T cell function. On the other hand, CTLA4-bound to B7-1 and B7-2 induces the expression of indoleamine 2,3-dioxygenase (IDO), which can promote the development of Treg cells<sup>53</sup>. Similarly, when PD1 on T cells binds to PD-L1 (B7-H1), it transduces inhibitory signals into T cells leading to suppressed T cell function. Additionally, PD-L1 expressed on cancer cells has been shown to transmit an anti-apoptotic signal from bound PD1<sup>54,55</sup>.

### III. Immune dysfunction in multiple myeloma

As shown in Figure 1, patients with multiple myeloma show profound defects/dysfunction in both innate and adaptive immunity<sup>56</sup>. The human innate immune system includes natural killer (NK) cells, macrophages, monocytes, neutrophils, eosinophils, and basophils. NK cells recognize and kill myeloma cells by activating receptors including NKG2D, DNAX accessory molecule (DNAM-1 or CD226) and the natural cytotoxicity receptors (NCRs) Np46, Np30, Np44<sup>57,58</sup>. NK cells play an important role in tumor immune-surveillance. However, in patients with active myeloma, the expression of these activating receptors on NK cells are reduced, leading to impaired NK cell activity<sup>59,60</sup>. Additionally, PD-1 expression is upregulated on NK cells; this allows PD-L1 expressing MM cells to inhibit the cytotoxicity of NK cells through the PD-L1/PD-1 pathway<sup>61</sup>. Macrophages are increased in the bone marrow microenvironment of patients with myeloma and support the survival and proliferation of multiple myeloma cells through both contact dependent and contact independent activation of the pro-proliferative molecule STAT3<sup>62,63</sup>. Neutrophils in patients with MM demonstrate reduced phagocytic activity and up-regulation of Arg-1 expression leading to an immunosuppressive effect on T lymphocytes<sup>64</sup>. Eosinophils have been shown to promote human and mouse myeloma cell growth<sup>65,66</sup>, and may in fact play a role in the biology and pathology of MM.

B and T lymphocytes mediate adaptive immunity. In MM patients, B-cell dysfunction is characterized by hypogammaglobulinemia, leading to an increased risk for infections. T-cell mediated immunity is also severely impaired in MM. MM patients demonstrate a decrease in the ratio of CD4(+)/CD8(+) T-cells in the peripheral blood: this is due to a decrease in both the absolute and relative numbers of CD4(+) T-cells, and an increase in relative numbers of CD8(+) T-cells<sup>67,68</sup>. Similarly, an increased Th1/Th2 ratio has been observed in MM patients and is associated with immune dysfunction<sup>69,70</sup>. Th17 cells—a pro-inflammatory subset of T-cells—are increased in both the peripheral blood and bone marrow of MM patients<sup>71,72</sup>. Th17 cells are particularly enriched in the bone marrow and produce significant levels of interleukin (IL)-17, which is implicated in the development of MM lytic bone lesions<sup>72</sup>. The number of immunosuppressive cells—including regulatory T and B cells and myeloid derived suppressive cells—are also significantly increased in MM patients<sup>56,73–75</sup>. T-cell mediated cytotoxicity depends upon the antigen presenting function of dendritic cells (DCs). The function of DCs is inhibited in MM patients due to the secretion of cytokines such as IL-6, IL-10 and TGF- $\beta$  by myeloma cells<sup>76,77</sup>. Additionally, numbers of plasmacytoid and myeloid DCs are decreased, and levels of PD-L1 expression

on plasmacytoid DCs are increased, resulting in T cell inhibition. Myeloid derived suppressor cells (MDSC) are a heterogeneous population of immature myeloid cells that are characterized by the ability to suppress anti-tumor immune responses mediated by T cells and NK cells<sup>78</sup>. There is a substantial increase in MDSCs in the peripheral blood and bone marrow of MM patients, which has been shown to play a role in disease progression and drug resistance<sup>79–81</sup>. MDSCs also promote tumor angiogenesis and growth by the secretion of cytokines and growth factors<sup>81</sup>. Notably, the percentage of PD-L1+ bone marrow MDSCs in myeloma patients in remission is unchanged from PD-L1 levels at diagnosis or at relapse; this suggests that, even with successful treatment, the bone marrow of MM patients continues to be immunosuppressed<sup>82</sup>. NKT cells express both T cell receptors and NK cell surface antigens and recognize glycolipids through CD1d. In MM patients, NKT cell's ability to produce IFN- $\gamma$  is defective leading decreased innate and adaptive immune activation<sup>83</sup>. Another T-cell subset, the  $\gamma\delta$  T cells, proliferate in the presence of IL-2 and exhibit anti-myeloma activity; their activity is also impaired in myeloma patients.

Immune dysfunction in myeloma patients is caused by several factors. The growth of the tumor within the bone marrow leads to a collapse of normal hematopoiesis. Myeloma cells produce several factors—transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-10, IL-6, and prostaglandin E2—which have a negative impact on the function of various immune cells<sup>84</sup>. For example, TGF- $\beta$  induces phosphorylation of SMAD3 and suppresses IFN- $\gamma$  production and antibody-dependent cellular cytotoxicity (ADCC). In addition, myeloma cells release major histocompatibility complex class I-related chain molecule A (MICA) into serum as the disease progresses. MICA functions as a ligand to the NKG2D receptor on  $\gamma\delta$  T cells, NK cells and CD8+ T cells which further dampens anti-myeloma immunity. Furthermore, lactase derived from myeloma cells upregulates VEGF through HIF-1 $\alpha$ , which directs tumor-associated macrophages (TAMs) to differentiate to an M2-like phenotype, leading to increased tumor angiogenesis and proliferation. M2-like macrophages are the dominant form of TAMs in the bone marrow of patients with myeloma<sup>85</sup>.

The bone marrow microenvironment can also contribute to immune dysfunction in myeloma patients. Bone marrow stroma cells can produce TGF- $\beta$  to suppress immune responses. Mesenchymal stem cells in myeloma patients exhibit abnormal expression of CD40/40L, VCAM1, ICAM-1, LFA-3, HO-1, HLA-DR and HLA-ABC and cause a shift in the Th17/Treg balance<sup>86</sup>. Osteoclasts produce galectin-9 (Gal-9) and a proliferation-induced ligand (APRIL), which lead to the inhibition of antitumor T cell responses through the Tim-3/Gal-9 pathway and PD-1/PD-L1 pathway<sup>87</sup>.

#### IV. CTLA4/CD28 and PD-1/PD-L1 pathways in multiple myeloma

##### CTLA-4

CTLA-4 is an inhibitory receptor expressed on T cells. The biological role of CTLA-4 is regulation of T cell responses, predominantly during initial activation in the lymph node and the prevention of autoimmunity; this has previously been illustrated by the development of massive lethal lymphoproliferation in CTLA-4 knock-out mice<sup>88,89</sup>. Recognizing the role of CTLA-4 as a negative regulator of immunity, investigators have shown that antibody blockade of CTLA-4 could result in antitumor immunity in preclinical models<sup>90</sup>. This led to

the development of ipilimumab (Yervoy, Bristol-Myers Squibb) and tremelimumab which are fully humanized monoclonal antibodies targeting CTLA-4. Ipilimumab has received FDA approval for the treatment of various solid malignancies, both as a single agent and in combination with anti-PD-1 targeting therapies.

### PD-1/PD-L1 Pathway

PD-1 (alias CD279), a member of the CD28 family of receptors, is expressed on the surface of antigen-activated T and B cells<sup>91</sup>. While NK cells normally do not express PD-1, NK cells from myeloma patients have been shown to express PD-1<sup>61</sup>. PD-L1 (B7 homologue-1) (B7-H1) is also expressed on myeloma cells, and this level of expression is higher in the plasma cells from MM patients compared with cells from MGUS patients and healthy volunteers<sup>92</sup>. PD-L1 expression is often up-regulated at relapse or in the refractory phase<sup>93,94</sup> and in patients who are positive for minimal residual disease<sup>95</sup>. Several groups have shown that PD-L1 expression is absent in normal plasma cells, but is expressed in myeloma cell lines and primary myeloma cells from patients with MM<sup>92,96</sup>. Compared to PD-L1 expressing myeloma cell lines, myeloma cell lines that do not express PD-L1 are more sensitive to chemotherapy and have lower levels of Bcl-2 and FasL expression<sup>93</sup>. PD-L1 expression on myeloma cells is further upregulated following *in vitro* treatment with IFN- $\gamma$  and TLR ligands through a common pathway involving MEK/ERK and MyD88<sup>97</sup>. PD-L1 expression on MM cells is also upregulated by the IL-6 signal cascade through activation of JAK2, STAT3, and MEK1/2. IFN- $\gamma$ , which is produced by CTLs and NK cells, is a strong inducer of PD-L1 expression in MM cells through the activation of the MEK/ERK pathway<sup>97,98</sup>. Finally, PD-L1 is expressed on myeloid and plasmacytoid DCs, MDSCs and nonhematopoietic cells located in the bone marrow microenvironment<sup>99</sup>.

As shown in figure 2 PD-L1 binds to PD-1 and delivers an inhibitory signal, reducing cytokine production and proliferation of T cells<sup>100</sup>. The binding of PD-1 to PD-L1 or PD-L2 decreases secretion of Th1 cytokines, inhibits T-cell proliferation, results in T-cell apoptosis, and inhibits CTL-mediated killing. Additionally, PD-1/PD-L1 binding has been shown to promote resistance to melphalan and bortezomib in myeloma cell lines through activation of the PI3K/AKT pathway<sup>101</sup>. A soluble form of PD-L1 produced through proteolytic cleavage of membrane-bound proteins from myeloma cells has recently been detected in some MM patients; higher levels of soluble PD-L1 in this population has been associated with poorer progression free and overall survival<sup>102,103</sup>.

## V. Preclinical studies targeting CTLA4/CD28 and PD-1/PD-L1 pathways

Immunosuppression is an important characteristic of MM pathology. Reversing this suppression could potentially restore myeloma immunosurveillance and improve disease control. Immune checkpoints are negative immunologic regulators that downregulate the magnitude of immune responses in order to protect the host from autoimmunity or damage from inflammation. This mechanism is frequently subverted by malignant cells, which escape immune surveillance by increasing inhibitory immune checkpoint ligands leading to host T cell exhaustion. Immune checkpoints have, therefore, become important therapeutic targets. Immune checkpoint inhibitors enhance the cytotoxic activity of host T cells by

blocking the inhibitory signals from tumor cells. Rather than targeting the cancerous cells directly, these agents stimulate the host's immune system to exert an antitumor effect<sup>104</sup>. The most clinically relevant checkpoints to date are CTLA-4 and PD-1/PD-L1 pathways. CTLA-4 is primarily believed to regulate immune responses early in T-cell activation while PD-1 is believed to inhibit T-cell activity in the effector phase within tissues and tumors. The use of antibodies to disrupt the receptor-ligand interactions involved in these pathways has shown remarkable results in several solid cancers (reviewed in<sup>105</sup>), and, more recently, in selected hematologic malignancies<sup>106–109</sup>.

## VI. Clinical studies targeting CTLA4/CD28 and PD-1/PD-L1 pathways in multiple myeloma

### Single agent activity

To date, there are no published clinical trials looking at ipilimumab or tremelimumab in MM patients. Ipilimumab was tested in a phase I trial of 28 patients with relapsed hematologic malignancies after allogeneic stem cell transplant. This trial included 1 MM patient who presented with pulmonary plasmacytomas. Overall, 5 of the 22 (23%) patients who received the maximum tolerated dose of ipilimumab (10mg/kg) had a complete response while another 2 (9%) patients had a partial response including the MM patient. Notably, the response in the MM patient was durable lasting for 21 months<sup>110</sup>.

Pidilizumab (CT-011), a humanized antibody blocking PD-1, was given as a single agent in 17 patients with advanced hematologic malignancies such as AML and B-cell lymphoma in a phase I study<sup>111</sup>. Patients were treated with one of 5 dose levels - 0.2, 0.6, 1.5, 3 and 6 mg/kg. CT-011 was safe and well tolerated in this patient population with no single maximum tolerated dose defined<sup>111</sup>. The median  $t_{1/2}$  of CT-011 ranged from 217 to 410 hours (9–17 days) and sustained increase in the percentage of peripheral blood CD4+ cells was observed for up to 21 days after treatment. Clinical benefit with a single administration of CT-011 was found in 33% of the patients, with one complete remission. Only 1 myeloma patient was enrolled in the study; this patient received the 6mg/kg dose and achieved stable disease for >13 months.

Single agent nivolumab (Opdivo, Bristol-Myers Squibb) was administered at doses of 1 or 3 mg/kg every 2 weeks in a phase I study in patients with relapsed or refractory B-cell lymphoma (n = 31), T cell lymphoma (n = 23), or multiple myeloma (n = 27)<sup>112</sup>. Nivolumab was well tolerated with only 34% of patients experiencing immune-mediated AEs that were predominately grade 1 or 2. Only half of the patients with immune mediated AEs required immunosuppression or interruption of nivolumab. Notably, the rate of all AEs and grade 3 or 4 AEs were lower in the MM patients (52% and 19% respectively) compared to the lymphoma patients (72% and 24% respectively)<sup>112</sup>. One patient with B-cell small lymphocytic lymphoma died of pneumonitis 19 days after receiving her initial dose of nivolumab. While nivolumab therapy resulted in an objective response rate of 36% and 40% among patients with DLBCL and FL, respectively, the best response for patients with MM was stable disease, which occurred in 17 (63%) MM patients and lasted a median of 11.4 weeks (range, 3.1 to 46.1 weeks)<sup>112</sup>.



Ribrag et al reported on the phase Ib KEYNOTE-013 study which tested the efficacy of pembrolizumab (Keytruda, Merck and Company) monotherapy for patients with relapsed/refractory multiple myeloma who had failed 2 prior lines of therapy including a proteasome inhibitor and an IMiD<sup>113</sup>. Thirty patients were treated with pembrolizumab 10mg/kg every 2 weeks or 200mg fixed dose every 3 weeks. At a median follow-up of 15 months, 28 (93%) patients discontinued the study due to disease progression. No patient experienced a response, and the best response observed was stable disease. Only 1 (3%) patient had an immune-related adverse event (grade 1 pruritis) and grade 3 treatment related AEs (myalgia) occurred in only 1 (3%) patient. There were no grade 4 TRAEs or deaths due to TRAEs<sup>113</sup>. These data are summarized in table 1.

### Combination therapy.

In preclinical studies, IMiDs were found to enhance the effects of PD-1/PD-L1 inhibitors on T cell- and NK cell- mediated cytotoxicity<sup>114</sup>. The combination of IMiDs and PD-1/PD-L1 inhibitors led to increased cell death of MM cells<sup>61,114,115</sup>. Notably, this effect was not associated with an increase in PD-1/PD-L1 expression on the effector cells<sup>114,116</sup>. These encouraging preclinical results led to clinical trials investigating the combination therapy of PD-1/PD-L1 inhibitors with IMiDs in patients with multiple myeloma which are summarized in table 2.

Because nivolumab continues to bind to the T cell PD-1 receptor for over 2 months after administration, Pianko et al recently reported on the efficacy and toxicity of therapy immediately after treatment with nivolumab in 19 relapsed myeloma patients<sup>117</sup>. Twelve of the patients were treated with FDA-approved standard therapies after nivolumab and 7 patients were given experimental therapy or enrolled on another clinical trial. Interestingly, of the 12 patients receiving standard therapies, 7 (58%) achieved a PR or better, 2 (17%) achieved a VGPR, and 1 (8%) patient who received radiation therapy for a plasmacytoma reached a CR that lasted for >30 months<sup>112,117</sup>.

Badros et al reported on a single-center, phase II study of the combination of pembrolizumab, pomalidomide and dexamethasone in relapsed/refractory MM. A total of 48 patients were enrolled in the study. Patients had previously received both an IMiD and proteasome inhibitor and had a median of 3 lines of prior therapy. Furthermore, 70% of enrolled patients had received an autologous hematopoietic stem cell transplant and 62% had high-risk cytogenetics. Pembrolizumab was given at 200mg IV every 2 weeks, pomalidomide 4mg daily 21 days on and 1 week off, and dexamethasone 40mg weekly in 28-day cycles. Overall, 60% of treated patients showed objective responses including CR in 8% of patients, VGPR in 19%, and PR in 33% of patients. Median duration of response was 14.7 months. At median follow up of 15.6 months, progression-free survival was 17.4 months. Grade 3–4 adverse events occurred in 42% of patients including neutropenia in 42% of patients, anemia in 21% of patients, hyperglycemia in 21% of patients and pneumonia in 15% of patients. Pneumonitis occurred in 13% and hypothyroidism in 10%, but grade 3 or 4 levels were only seen in 2% and 4% respectively. Analyses of pretreatment bone marrow samples revealed a non-statistically significant trend ( $p = 0.06$ ) for increased expression of

PD-L1 in responders and a moderately statistically significant positive correlation between PFS and increased T-lymphocyte infiltrates, irrespective of PD-1 expression ( $p=0.05$ )<sup>118</sup>.

Keynote-023 (NCT02036502) was a phase I trial that tested the combination of pembrolizumab, lenalidomide, and low-dose dexamethasone in patients who had previously progressed on 2 prior therapies including a proteasome inhibitor and an IMiD. As of May 2017, a total of 62 patients with RRMM were enrolled in the study and 50 patients were evaluable for response. The MTD/MAD was pembrolizumab 200 mg fixed dose in combination with lenalidomide 25 mg and dexamethasone 40 mg. Of the evaluable patients 22 (44%) responded to treatment with 2 (4%) patients achieving a sCR, 6 (12%) patients achieving a VGPR, and 14 (28%) patients achieving a PR. Additionally, another 25 (50%) patients met criteria for SD for an overall disease control rate of 94% in the relapsed refractory setting<sup>119</sup>. Responses were durable, with a median duration of 18.7 months in the responders. Interestingly, while the overall PFS of the patients who were lenalidomide refractory was shorter (6.3 vs 7.2 months), duration of response was significantly longer at 24.9 months in the 13 patients who were lenalidomide refractory and achieved a PR or better. Only 8 patients (13%) experienced immune mediated adverse events (most commonly thyroid dysfunction), but there were 2 deaths in the trial: one from hepatic venoocclusive disease, and one from ischemic stroke<sup>119</sup>. This data suggests that PD-1 blockade with pembrolizumab in combination with lenalidomide and dexamethasone is associated with a tolerable safety profile and promising antimyeloma activity in heavily pretreated patients with RRMM.

The Keynote-183 phase III trial randomized patients with relapsed/refractory multiple myeloma who had received at least 2 lines of prior treatment to receive pomalidomide and low-dose dexamethasone with or without pembrolizumab (200 mg every 3 weeks). At the data cutoff of June 2, 2017, a total of 249 patients were enrolled: 125 randomized to the pembrolizumab arm and 124 patients randomized to pomalidomide and dexamethasone alone. The objective response rate (ORR) was 34% versus 40% in the pembrolizumab and control arms, respectively. The median time-to-progression (TTP) was 8.1 months versus 8.7 months, respectively (HR, 1.14; 95% CI, 0.75–1.74)<sup>120</sup>. At the median follow-up of 8.1 months, there were 29 deaths in the pembrolizumab arm versus 21 deaths in the control arm, with the pembrolizumab group showing a greater than 50% increase in the relative risk of death compared with the control arm. The rate of severe, grade 3–5 toxicity was 83% in the pembrolizumab arm versus 65% in the control group. The rates of serious adverse events (AEs) were 63% versus 46%, respectively. Causes of death unrelated to disease progression in the pembrolizumab cohort included myocarditis, Stevens-Johnson syndrome, myocardial infarction, pericardial hemorrhage, cardiac failure, respiratory tract infection, neutropenic sepsis, sepsis, multiple organ dysfunction, and respiratory failure<sup>120,121</sup>.

Similarly, the Keynote-185 phase III trial randomized newly diagnosed, treatment naïve MM patients who were ineligible for autologous stem cell transplant to receive lenalidomide and low-dose dexamethasone with or without pembrolizumab (200 mg every 3 weeks). A total of 301 patients were included in the safety and efficacy analysis at the data cutoff date of June 2, 2017. The ORR was 64% in the pembrolizumab arm versus 62% in the control arm. The median TTP had not yet been reached in either arm (HR, 0.55; 95% CI, 0.20–1.50) at the

time of data cutoff. At the median follow-up of 6.6 months, there were 19 deaths in the pembrolizumab arm compared with 9 deaths in the control arm (HR for OS, 2.06; 95% CI, 0.93–4.55). The relative risk of death in the pembrolizumab arm was more than double that of the control group. The rate of severe, grade 3–5 toxicity was 72% in the investigational arm versus 50% in the control arm. The rates of serious AEs were 54% versus 39%, respectively. Causes of death unrelated to disease progression identified in the pembrolizumab cohort included intestinal ischemia, cardiorespiratory arrest, suicide, pulmonary embolism, cardiac arrest, pneumonia, sudden death, myocarditis, large intestine perforation, and cardiac failure<sup>121,122</sup>. Because of the increased risk for death observed in pembrolizumab arm in both the Keynote-183, and 185 studies, the FDA halted these trials on July 3, 2017 (Merck, the manufacturer of Pembrolizumab, had ceased enrolling new patients as of June 12, 2017).

Development of immune-related adverse events (irAEs) is associated with response to checkpoint inhibitors in some disease settings<sup>123–126</sup>. However, data from Keynote-183 and Keynote-185 yielded inconsistent results regarding the relationship between irAEs and response to checkpoint inhibition. As reported by Krauss et al. at the 2018 ASCO annual meeting, the Keynote-183 trial had 70 patients (56%) in the pembrolizumab, pomalidomide and dexamethasone arm develop an irAE. These patients had an ORR of 37%, which was not significantly different from the 31% ORR in the 55 patients (44%) who did not have irAEs. Of the 21 patients that experienced Grade 3 irAEs in the pembrolizumab containing arm, only 6 (29%) achieved a PR or better. These data suggest no association between irAEs and ORR. However, in Keynote-185, 102 patients (68%) in the pembrolizumab containing arm had one or more irAEs, and this was associated with an ORR of 73%. Response rate in the 54 patients (36%) who had grade 3 irAEs was similar at 70%. Both of these values are significantly higher than the response rate in the 49 patients who did not have any irAEs (45%), suggesting an association between response and irAEs in this trial<sup>121</sup>. The reason for these disparate findings remains unclear. One possibility is that patients with newly diagnosed myeloma, such as those in the Keynote-185 study, retain greater capacity to mount an immune response by virtue of having not been exposed to the dampening effects of prior therapy. Interestingly, both of these studies showed significant irAEs for their respective control arms (45% in Keynote183, and 44% in Keynote-185) which seems to correlate with increased response<sup>121</sup>. These data confirm that IMiDs are indeed immunomodulators and that their ability to induce an immune response is preserved in both newly diagnosed and relapsed refractory MM.

Combinations therapy with nivolumab in MM patients is currently being investigated in several clinical trials. Checkmate-039 is a phase I study that aims to establish the tolerability of nivolumab and the combination of nivolumab and daratumumab, with or without an IMiD (pomalidomide and dexamethasone) in subjects with relapsed or refractory MM<sup>127</sup>. CA204142 is a phase II, multiple cohort study of elotuzumab in combination with pomalidomide, low-dose dexamethasone, and nivolumab, in patients with relapsed or refractory multiple myeloma who have previously progressed on a lenalidomide containing regimen. Checkmate-602 is a phase III, open-label, randomized trial evaluating the combination of nivolumab, elotuzumab, pomalidomide, and dexamethasone for patients with relapsed/refractory multiple myeloma<sup>128</sup>. Checkmate-602 is supported by preclinical models

which have shown that the combination of elotuzumab and anti-PD-1 resulted in increased tumor-infiltrating NK cells and activation of CD8+ T-cells<sup>129</sup>. All 3 trials were placed on partial clinical holds in October 2017 by the FDA as part of the review of the data on pembrolizumab. On December 5, 2017, the holds placed on the phase I CheckMate-039 and phase II CA204142 trials were lifted by the FDA; while the partial hold on the phase III CheckMate-602 trial study remained in place until June 2018.

The MEDI4736-MM-003 trial (NCT02807454) was designed to study the efficacy of the PD-L1 inhibitor Duvalumab (Iminzi, Astra-Zeneca) in combination with daratumumab with and without pomalidomide and dexamethasone in RRMM<sup>130</sup>. However, the FDA placed this trial on partial hold in September 2017; the study was terminated shortly thereafter. Only 37 patients were enrolled in the study at the time of discontinuation. A phase I study (NCT02716805) to assess the safety and tolerability of tremelimumab and durvalumab administered with high dose chemotherapy and autologous stem cell transplant in high risk MM was also terminated after only 6 patients had been enrolled.

The investigational PD-1 inhibitor JNJ-63723283 was being tested in combination with daratumumab in the MMY-2036 study (NCT03357952) in patients with relapsed refractory MM who had received 3 lines of prior therapy, including an IMiD and proteasome inhibitor. At the same time, the combination of daratumumab and the PD-L1 inhibitor atezolizumab (Tecentriq, Roche/Genentech) was being tested in NSCLC (NCT0302342). Interim analysis of this trial by the data monitoring committee suggested no improvement in efficacy with the combination compared to atezolizumab alone, and increased rate of mortality in the combination arm. Based on these results GenMab announced in May of 2018 that Janssen would stop studies of daratumumab in combination with anti-PD-1/PD-L1.

## V. Future directions in treatment with immune checkpoint inhibitors

As detailed above, checkpoint inhibitors have proven challenging in the treatment of multiple myeloma. Targeting the CTLA4 and/or PD-1/PD-L1 pathways can re-activate and boost antitumor immunity, resulting in disease regression and prolonged survival in solid tumors. However, checkpoint inhibitors have not been effective as a single agent in multiple myeloma patients. Furthermore, current combinations of checkpoint inhibitors with IMiDs have yielded disappointing results and were associated with an increased risk of death in randomized phase III studies. While checkpoint inhibitors remain an attractive therapy for MM, further research is needed to improve efficacy and limit toxicity with these agents. We proposed three main areas for future research endeavors.

### 1. A comprehensive understanding of the unique immune dysfunctions in multiple myeloma.

Unlike solid tumors, multiple myeloma is a malignancy of plasma cells which produce monoclonal immunoglobulin proteins. These plasma cells and abnormal immunoglobulin proteins cause profound immune dysfunction that is unique to MM. For instance, it was reported that T cells in multiple myeloma demonstrated senescent phenotypes rather than the exhausted phenotypes typically seen in solid tumors<sup>131</sup>. Additionally, 75% of myeloma

patients showed clonal expansion of T cells that express low levels of PD-1 or CTLA-4, but also express markers consistent with telomere-independent senescence<sup>132</sup>. The presence of senescence demonstrates the need to evoke the expansion of activated, myeloma-reactive T cells and provides a rationale for combination therapy. Similarly, it is very likely that the functions and phenotypes of other immune cells such as Tregs, MDSCs, and NK cells could be different in multiple myeloma patients when compared to patients with other malignancies. In conclusion, a more thorough understanding of the unique immune dysfunctions of myeloma patients is warranted.

Secondly, the repertoire of co-signaling molecules on T cells, antigen presenting cells, and tumor cells is highly versatile and context dependent. The potential effects of administration of CTLA4 or PD-1/PD-L1 antibodies could have on the expression and levels of other co-signaling molecules are largely unknown. It is possible that administration of CTLA4 antibody and PD1/PD-L1 antibodies could lead to compensatory up-regulation of other immune checkpoints (as has been seen in other cancers)<sup>133,134</sup>, or increases in MDSCs and/or Tregs cells.

Finally, many other factors can impact the efficacy of CTLA4 or PD-1/PD-L1 targeted therapy. For instance, the responses to PD-1 targeted therapy are quite different among relapsed/refractory MM, newly diagnosed MM, and smoldering MM patients. Factors such as patient population (age, co-morbidities, disease), prior therapy, and history of stem cell transplant can affect CTLA4 or PD-1/PD-L1 targeted therapy.

Ratner et al. recently reported rapid progression of adult T cell leukemia/lymphoma after antiPD-1 therapy in the first three patients of their phase II trial<sup>135</sup>. Interestingly, Ishitsuka et al. performed a similar phase II trial in Japan with 8 patients who were treated with at least one dose of nivolumab and none of these patients showed rapid disease progression<sup>136</sup>. It was postulated that these conflicting results might be caused by differences in the patients' disease status and therefore a difference in the immune environment. The 3 patients in Ratner's report had ATLL with smoldering or very slow progression. In contrast, the patients in the Japan cohort had aggressive disease or chronic type with unfavorable prognostic factors. These findings suggest that disease status and the immune environment may play a role in PD-1 treatment.

Recently, it was shown that gut microbiome and antibiotic use affect the efficacy of PD-1 based immunotherapy against epithelial cancers (melanoma, sarcoma, non-small cell lung cancer, and renal cell carcinoma)<sup>137-139</sup>. Patients with advanced NSCLC, RCC or urothelial carcinoma who were prescribed antibiotics within 2 months before or 1 month after the first administration of PD-1/PD-L1 antibody demonstrated a shorter PFS and OS<sup>137</sup>.

Metagenomics of patient stool samples revealed a correlation between treatment responses to checkpoint inhibitors and the relative abundance of *Akkermansia muciniphila*. The presence of *A. muciniphila* was associated with increased recruitment of tumor infiltrating CCR9+CXCR3+CD4+ T lymphocytes and enhanced efficacy of PD-1 blockade in an IL-12 dependent manner. When feces from NSCLC patients who responded to anti-PD-1 therapy were transplanted into germ-free or antibiotic-treated mice, the animals had improved response to PD-1 blockade. Conversely, feces from patients who did not respond to anti-

PD-1 did not improve response to PD-1 blockade in germ-free mice. However, when these mice received oral *A. muciniphila* alone or combined with *Enterococcus hirae* (which stimulates goblet cells), tumor growth slowed<sup>137</sup>. Similarly, analysis of stool in melanoma patients prior to treatment with anti-PD-1 immunotherapy showed higher levels of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* in the responders. Once again, transfer of fecal material from the responders into germ free mice slowed the growth of implanted B16.SIY melanoma cells compared to mice who received fecal material from the non-responders<sup>138</sup>. It is very likely that chemotherapy and antibiotic use in myeloma patients could affect composition of the gut microbiome and diminish the efficacy of checkpoint inhibitors. These data need to be weighed against the recent data that showed a survival benefit with prophylactic antibiotic use in myeloma patients undergoing induction chemotherapy<sup>140</sup>.

## 2. Identification and development of effective combination therapies with checkpoint inhibitors.

Most of the combination chemoimmunotherapy trials to date examine combinations of checkpoint inhibitors with IMiDs. As previously noted, IMiDs reduce PD-1 expression on T cells and NK cells as well as PD-L1 expression on myeloma cells and MDSCs. Therefore, cotreatment with an IMiD and anti-PD1/PD-L1 could lead to reduced target antigens on the effector cells and thus less antibody binding. Furthermore, this downregulation of PD-1/PD-L1 could lead to a compensatory up-regulation of other immune checkpoints—a mechanism that has been previously described in other cancers<sup>133,134</sup>—which could contribute to the lack of activity seen with these agents in combination. Further investigation in this area is clearly warranted to explore other potential combinations of therapy that could capitalize on checkpoint inhibition.

**Combination of radiotherapy and anti-PD-1/PD-L1 antibody.**—It is noteworthy that only one patient achieved a CR with nivolumab single agent therapy<sup>112</sup>. This patient developed a rib plasmacytoma while on nivolumab. Nivolumab was stopped and the patient underwent local radiation. After completion of radiation therapy, the patient was restarted on nivolumab for 2 months and achieved a CR that lasted for >14 months<sup>112</sup>. This single case suggests that the combination of radiotherapy and anti-PD-1/PD-L1 monoclonal antibody may be useful for patients with MM; an effect that was previously reported in several solid cancers<sup>141–143</sup>. In these instances, treatment with radiation is hypothesized to convert tumors to a more immunogenic phenotype by upregulating antigenic expression and co-stimulatory molecules, by increasing the secretion and production of immunostimulatory cytokines, and by increasing recruitment of APCs and effectors into the tumor microenvironment<sup>144</sup>.

**Combination of tumor vaccines with checkpoint inhibitors.**—The addition of tumor vaccines to treatment with checkpoint inhibitors could prime antigen presenting cells and T cells for antitumor immunity. Rosenblatt et al administered a multiple myeloma vaccine made from patient-derived tumor cells fused with autologous dendritic cells to patients following AHSCT and demonstrated immunologic and clinical responses<sup>145,146</sup>. *In vitro* studies suggest that PD-1 blockade by pidilizumab (CT-011) acts synergistically when

co-administered with a DC/Myeloma fusion vaccine<sup>147</sup>. This combination is currently being evaluated in a phase II clinical trial following AHSCT<sup>148</sup>.

**Combination of chemotherapy with checkpoint inhibitors and using checkpoint inhibitors in the post autologous transplant setting.**—One of the major immune dysfunctions in myeloma patients results from the presence of immune suppressor cell populations such as Tregs and MDSCs which can persist even after patients achieve successful remission<sup>82</sup>. Therefore, one strategy to enhance the efficacy of checkpoint inhibitors is to use cytotoxic therapy to deplete immune suppressor cells thus enhancing the reconstitution of myeloma immunity<sup>149</sup>. Similarly, autologous hematopoietic stem cell transplant was reported to be able to deplete regulatory T cells and cause concurrent expansion of myeloma-specific clones<sup>145,150</sup>. Therefore, the combination of a checkpoint inhibitor with a DC/tumor vaccine following AHSCT has been hypothesized to enhance the efficacy of checkpoint inhibitors in myeloma<sup>148</sup>.

**Combination of CAR-T cells with checkpoint inhibitors.**—Chimeric antigen receptor (CAR)-T cell therapy has become a very promising treatment for relapsed/refractory MM. This therapy involves the infusion of large amounts of myeloma cell-specific effector T cells<sup>151</sup>. Early phase clinical trials have shown impressive efficacy in heavily pretreated, highly relapsed refractory patients<sup>152–154</sup>, but it remains to be seen whether these responses will be durable. Combination of CAR-T cell therapy with checkpoint inhibitors could lead to enhanced antimyeloma effects and increased duration of response. Indeed, preclinical models have demonstrated augmented anti-tumor activity with the combination of CAR-T cell therapy and a PD-1 antibody<sup>155</sup>. However, several potential concerns of combining these treatment modalities exist. These include: increased T-cell anergy or apoptosis of CAR-T cells when bound to CTLA4 or PD-1/PD-L1, and increased risk for adverse events such as cytokine release syndrome or CNS toxicity with overexcitation of immune effectors<sup>149,156</sup>.

**Combination of various checkpoint inhibitors.**—The immune response is regulated by highly dynamic, overlapping, and numerous coinhibitory molecules. It has previously been demonstrated that at 3 and 12 months after autologous transplant, CTLA-4, LAG3, and TIM-3 are expressed on T cells isolated from patients with MM<sup>150</sup>. A preclinical study indicated that blocking PD-L1 together with other immune checkpoints (CTLA-4, LAG-3 or TIM-3) promotes the survival MM-bearing mice following low dose total body irradiation<sup>157</sup>. Similarly, the inhibitory immunoreceptor T cell immunoglobulin and ITIM domains (TIGIT) have been shown to have greater expression in MM patients than PD-1. Recent data has shown that inhibition of TIGIT leads to significantly improved OS in animal models of MM compared with anti-PD-1<sup>158,159</sup>. Moving forward, it will be critical to understand the clinical effects of blocking these pathways alone and in combination with PD-1 blockade. Currently, the combination of CTLA4 and PD-1 inhibitors is being evaluated in a phase Ib/IIa trial of patients with diffuse large B-cell lymphoma, T-cell lymphoma, or multiple myeloma who are at high risk for relapse following autologous stem cell transplant (NCT02681302).

**Combination of COX2 inhibitors with anti-PD-1/PD-L1 antibody.**—The enzyme cyclo-oxygenase-2 (COX-2) catalyzes the synthesis of prostaglandin E2. Both COX-2 and PGE2 can directly mediate pro-tumor activities and recruit and induce MDSCs in the tumor microenvironment<sup>160</sup>. Several preclinical models have demonstrated that inhibition of COX-2 or PGE2 combined with PD-1 pathway blockade acts synergistically<sup>161–163</sup>. This suggests that COX inhibitors could be useful adjuncts for immune-based therapies, including PD-1 blockade in cancer patients. Similarly, entinostat, a selective class I and class IV histone deacetylase inhibitor, has been shown to significantly reduce arginase-1, iNOS and COX-2 levels and to neutralize MDSCs. The combination of entinostat and PD-1 inhibition yielded enhanced antitumor effects in murine models of lung and renal cell carcinoma compared to PD-1 inhibition alone<sup>164</sup>.

**Combination of interferon or interferon activator with checkpoint inhibitors.**—Interferon was the first drug used to stimulate the immune system. Its efficacy was only modest however, and it currently does not have a role in myeloma treatment. Recent publications highlight a potential role for interferon in combination with other immune modulators—such as checkpoint inhibitors. Specifically, it has been shown that ablation of histone demethylase LSD1 activates type I interferon, which stimulates anti-tumor T cell immunity. This elicits significant responses to checkpoint blockade in mouse melanomas which were previously refractory to anti-PD-1 therapy<sup>165</sup>. Similarly, the combination of interferon and anti-CTLA4 antibody had superior efficacy compared to anti-CTLA4 antibody alone in a phase Ib trial of melanoma patients<sup>166</sup>.

**Supplementation of gut microbiome with PD-1/PD-L1 treatment.**—It was recently demonstrated that the gut microbiome influences the efficacy of PD-1 antibody treatment in solid tumors<sup>137–139</sup>. How concurrent antibiotic use and the gut microbiome affect the efficacy of checkpoint inhibitors in multiple myeloma is largely unknown. It is possible that, similar to solid tumors, oral supplementation of *A. muciniphila* could restore or enhance the efficacy of checkpoint inhibitors in multiple myeloma; prior exposures to antibiotics could therefore be potentially deleterious to myeloma patients being considered for immunotherapy. However, this must be weighed against the potential benefit of prophylactic antibiotics which was recently demonstrated in a large phase III trial of newly diagnosed MM patients<sup>140</sup>.

### **3. Development of clinical/biomarkers for immune monitoring and predicting immune-related adverse events.**

Looking forward, it will be critical to develop clinical/biomarkers that can be used to monitor immune responses during checkpoint inhibitor treatment, predict treatment responses to checkpoint inhibitors, and detect irAEs early before they become severe. In solid cancers, neoantigens, MHC antigens, PD-1 expression level, microsatellite instability, and mutational load have been proposed to correlate with treatment response<sup>167</sup>. Tumor necrosis factor, interferon-gamma, and various cytokines were found to be predictive markers for PD-1 treatment in advanced non-small cell lung cancer<sup>168</sup>. Level of peripheral blood eosinophils, IL-17 level, gene expression profiling, and digestive infiltrate by



neutrophils have been found to be associated with checkpoint inhibitor toxicities in solid malignancies<sup>169</sup>.

## VII. Conclusions.

Multiple myeloma remains an incurable disease. Immunotherapy using checkpoint inhibitors holds great promise in reversing the suppressive anti-myeloma immune response in MM patients. Thus far, the efficacy and safety profile of checkpoint inhibitors have been disappointing in MM. However, a full understanding of the immune defects present in the MM patient is required to appreciate the unique state of immune impairment that this disease entails. With this understanding, new combinations of agents and novel targets of immune therapy can be nominated to provide innovative therapies for this disease. Adoptive T cell transfer therapy has demonstrated very promising efficacy in myeloma treatment.

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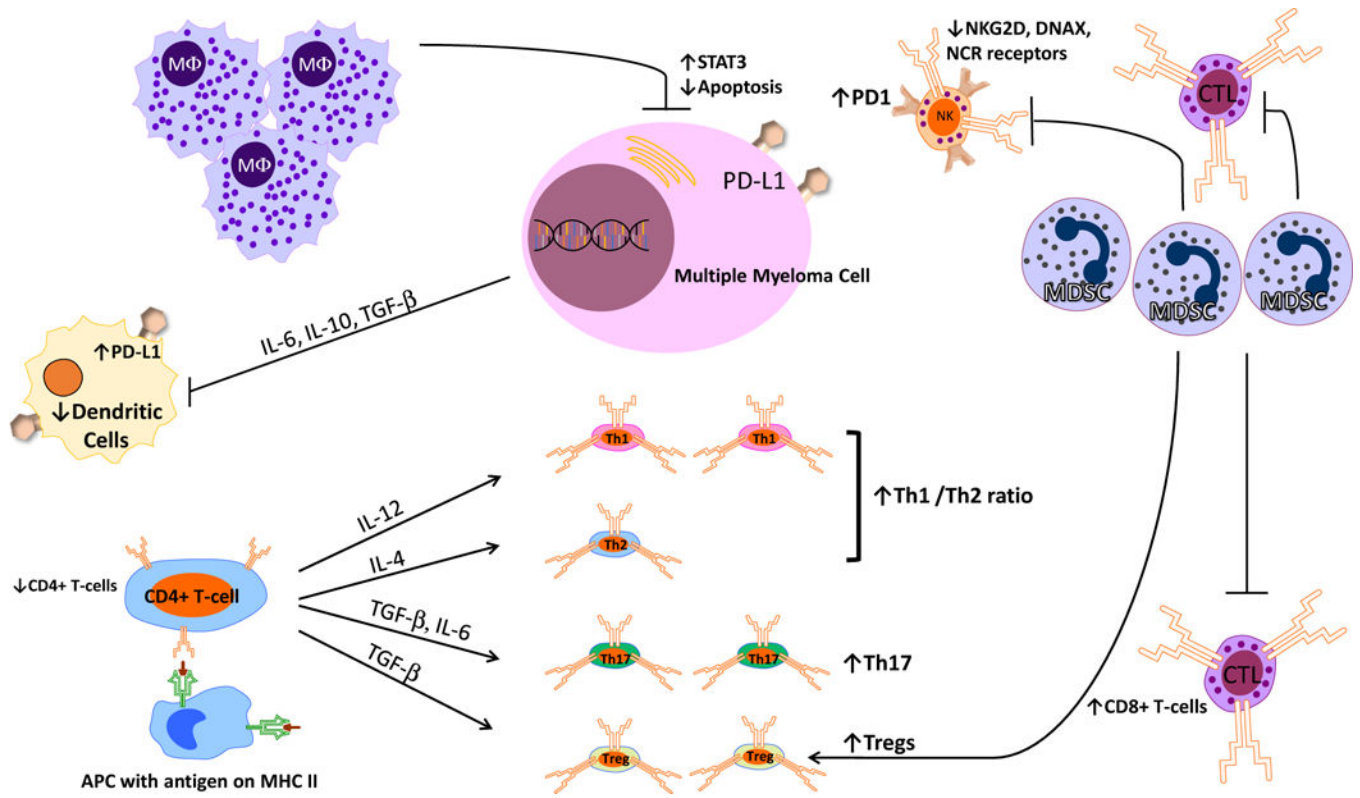


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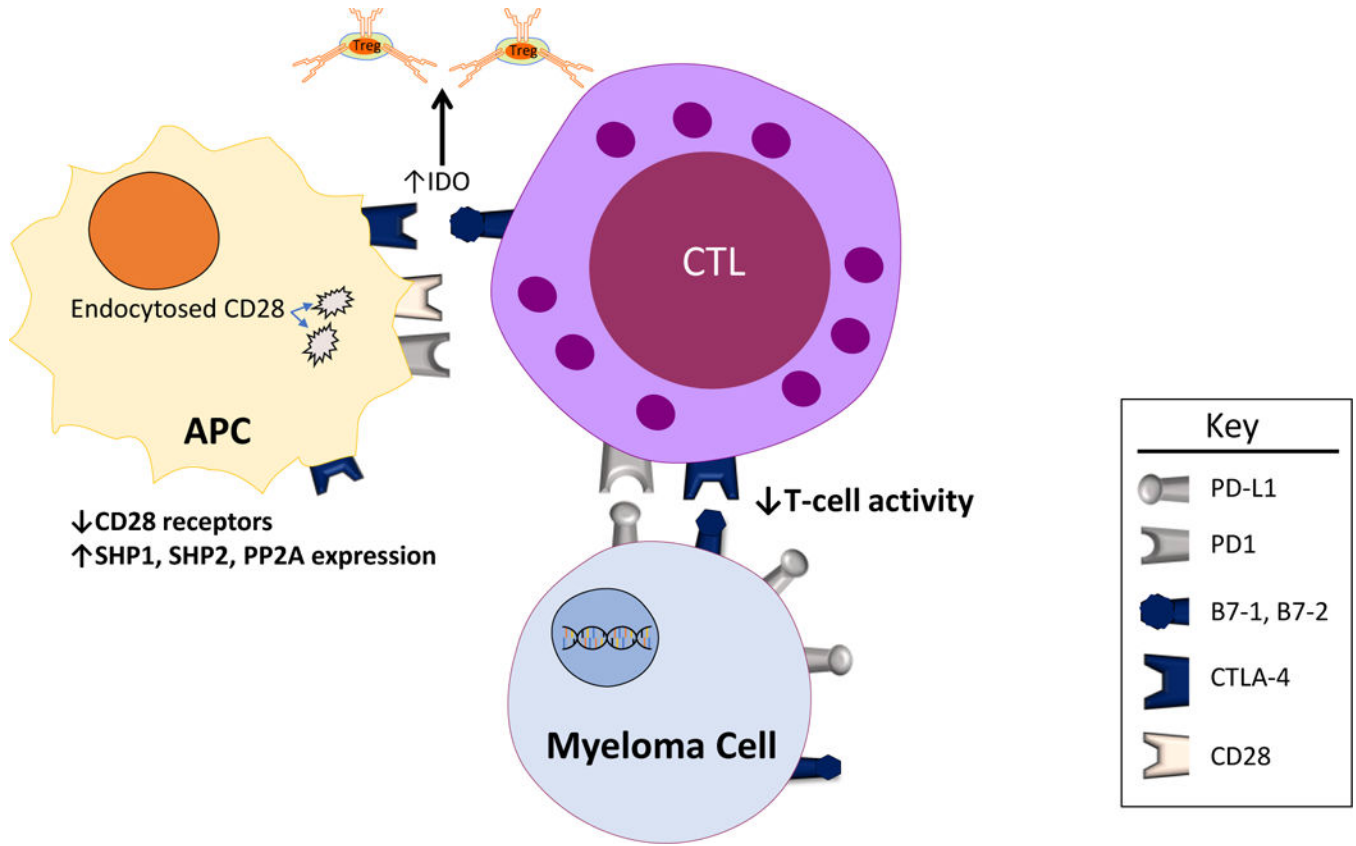
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### Highlights

Multiple myeloma is associated with profound defects/dysfunction in both innate and adaptive immunity. PD-L1 expression is up-regulated in myeloma cells and at relapse or in the refractory phase, which results in the suppression of cytotoxic T cell activity. While checkpoint inhibitors are effective in prolonging overall survival in some patients with advanced solid cancers and Hodgkin lymphoma, they have not demonstrated significant anti-myeloma activities as a single agent in MM. The combination of checkpoint inhibitors with immunomodulatory agents was recently found to increase the risk of death in myeloma patients. Additional studies are needed for a better understanding of immune dysregulation in myeloma patients, and the mechanisms of action of- and resistance to- checkpoint inhibitors



**Figure 1:**  
Immune Dysfunction in Multiple Myeloma (see text for details)



**Figure 2:** Effects of Immune Checkpoints in Myeloma. Binding of costimulatory molecules (B7-1, B7-2, or PD-L1) on myeloma cells to their respective receptors on dendritic cells results in downstream decreased immune activation. Specifically, expression of the phosphatases SHP1, SHP2, and PP2A are increased, and levels of the CD28 receptor (which is increases immune activation when bound to B7-1 or B7-2) are decreased via endocytosis of the cell surface receptors. Binding of B7-1 or B7-2 on cytotoxic T-cells with CTLA-4 on dendritic cells results in increased IDO expression which leads to increased levels of the immunosuppressive Treg cells.

**Table 1:**

Summary of Single Agent Checkpoint Inhibitor Trials. (ORR: overall response rate, DC: disease control rate, PR: partial response, SD: stable disease).

Agent	Target	Trial Population	N (MM Patients)	Overall Endpoint	Response Rate (%)	# of Myeloma Responders(%)	Response Rate MM(%)	Best Response in MM patients	Ref
Ipilimumab	CTLA4	Relapsed Hematologic Malignancies	22 (1)	ORR	32	1	100	PR	108
Pdilizumab	PD-1	Advanced Hematologic Malignancies	17 (1)	DC	33	1	100	PR	109
Nivolumab	PD-1	Relapsed Refractory Hematologic Malignancies	81 (27)	DC	16	1	4	SD	110
Pembrolizumab	PD-1	Relapsed Refractory MM	30 (30)	ORR	0	0	0	SD	111

**Table 2:**

Summary of Combination Trials with Checkpoint Inhibitors. (ORR: overall response rate, sCR: stringent complete response, CR: complete response, irAEs: Immune related adverse events, NR: not reported).

Combination	N	ORR (%)	Best Response i	irAEs(%)	Grade 3–4 irAEs	Ref(s)
Pembrolizumab, Pomalidome, Dexamethasone	48	60	CR	33	10	116
Pembrolizumab, Lenalidomide, Dexamethasone	62	44	sCR	13	0	117
Pembrolizumab, Pomalidome, Dexamethasone	125	34	CR	58	18	118,119
Pembrolizumab, Lenalidomide, Dexamethasone	151	64	NR	68	44	119,120

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