PD-1/PD-L1 inhibitors in multiple myeloma: The present and the future

T. Jelinek\textsuperscript{a,b,c} and R. Hajek\textsuperscript{b}

\textsuperscript{a}Faculty of Science, University of Ostrava, Czech Republic; \textsuperscript{b}Department of Haematology, University Hospital Ostrava and Faculty of Medicine, University of Ostrava, Czech Republic; \textsuperscript{c}Centro de Investigacion Medica Aplicada (CIMA), Clinica Universidad de Navarra, IDISNA, Pamplona, Spain

ABSTRACT

The introduction of PD-1/PD-L1 pathway inhibitors has marked a significant milestone in the treatment of various types of solid tumors. The current situation in multiple myeloma (MM) is rather unclear, as distinct research groups have reported discordant results. This discrepancy dominantly concerns the expression of PD-1/PD-L1 molecules as well as the identification of the responsible immune effector cell population. The results of monotherapy with PD-1/PD-L1 inhibitors have been unsatisfactory in MM, suggesting that a combination approach is needed. The most logical partners are immunomodulatory agents as they possess many synergistic effects. We are also proposing other rational and promising combinations (e.g., daratumumab, ibrutinib, anti-CD137) that warrant further investigation.

Introduction

Multiple myeloma (MM) is a genetically heterogeneous clonal plasma cell disorder, the second most common hematologic malignancy, representing approximately 1% of all cancers.\textsuperscript{1,2} Treatment advances as well as rapid drug development has made MM one of the most progressive and exciting fields in medical oncology. Proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) have become the standard of care and have significantly improved survival rates of myeloma patients.\textsuperscript{3} New generations of these drugs (pomalidomide, carfilzomib, ixazomib) together with molecules possessing distinct mechanisms of action like monoclonal antibodies (daratumumab [anti-CD38], elotuzumab [anti-CS1]) and histone deacetylase inhibitors (panobinostat) have recently been approved by regulatory authorities.\textsuperscript{4-9}

Immunotherapy has been playing an increasingly important part in the treatment of a vast majority of solid and hematologic cancers. Its fundamental principles are represented by stimulating the patient’s own immune system to fight harder and smarter against the tumor or by inserting artificially modified immune cells specifically targeting malignant cells. Allogeneic stem cell transplantation is an antique and toxic example of cellular immunotherapy still maintaining its indication in a subset of myeloma patients.\textsuperscript{10} Chimeric antigen receptor (CAR) T cells, CAR expressing NK cells or dendritic cell vaccines represent other types of immunotherapy with promising results seen also in MM.\textsuperscript{11} Monoclonal antibodies (mAbs), dominantly targeting antigens on the surface of malignant plasmocytes, are the largest group of immunotherapeutic agents. Several well described mechanisms of action (ADCC—antibody-dependent cellular cytotoxicity, CDC—complement-dependent cytotoxicity, ADCP—antibody-dependent cellular phagocytosis and direct apoptosis) are responsible for killing targeted cells.\textsuperscript{12}

This review is dedicated to describe another group of mAbs targeting immune checkpoints. “Immune checkpoints” refer to a plethora of inhibitory or stimulatory pathways encrypted in the immune system that are crucial for self-tolerance and for the modulation of physiological immune responses. Inhibitory checkpoints, under normal conditions, are essential for the prevention of autoimmunity and for tissue protection from damage when the immune system is responding to a pathogenic infection.\textsuperscript{13} Nevertheless, these native pathways may be abused and hijacked by tumor cells that become invisible to the host’s immune system as they start to express ligands of checkpoint receptors on their surface.\textsuperscript{14}

PD-1/PD-L1 axis

Among many others, there are two major deeply explored inhibitory pathways: (i) cytotoxic T-lymphocyte associated protein 4 (CTLA-4, CD152) as a checkpoint receptor and its cognate ligands B7-1 (CD80) and B7-2 (CD86) and (ii) programmed-death 1 (PD-1, CD279) receptor with its two ligands PD-L1 (CD274 B7-H1) and PD-L2 (CD273, B7-DC). The PD-1 receptor is a 288 amino acid type I transmembrane protein, a part of the CD28 receptor family, expressed on antigen-activated and exhausted T and B cells.\textsuperscript{15,16} The engagement of the PD-1 receptor with its ligands PD-L1 or PD-L2 leads to temporary downregulation of T cell function, namely decreased T cell proliferation, cytokine production and cytotoxicity and increased susceptibility to apoptosis.\textsuperscript{14,17,18} This cascade leads to T cell exhaustion and immune escape which is originally well known in chronic viral infections such as hepatitis B, hepatitis C or HIV, where these changes protect the host from an excessive immune response.\textsuperscript{19,20}
Many solid and hematologic tumors use this strategy (over-expression of PD-L1 or 2) to escape from the host’s immune surveillance. The recognition of this immune evasion mechanism has led to the development of therapeutic mAbs directed against receptors or ligands involved in this pathway. This treatment modality has demonstrated an unprecedented practice-changing activity in solid oncology, with drugs having already been approved for melanoma, non-small cell lung cancer, renal cell carcinoma and head and neck cancer with probably many others soon to follow.21-24 Among hematologic disorders, it was only in classical Hodgkin’s lymphoma that the checkpoint inhibitors achieved remarkable results, leading to accelerated FDA approval in 2016.25 The aim of this review is to elucidate the role and potential importance of PD-1/PD-L1 inhibitors in the treatment of MM, as well as to describe and discuss all available preclinical and clinical results with these compounds and to offer insights for the future.

Expression of PD-L1 and PD-1 in multiple myeloma

Expression of PD-L1 on plasma cells

Numerous authors have reported that PD-L1 is expressed on pathological plasma cells (PCs) from myeloma patients but not on normal PCs from healthy donors (HD).26-30 Likewise, the expression of PD-L1 was reported as being higher on PCs in MM and smouldering MM (SMM) than in monoclonal gammopathy of undetermined significance (MGUS).26,31 Nevertheless, various investigators used different methodologies, different cut-offs of positivity, different gating strategies, and their results varied in the percentage of PD-L1 positive PCs [Liu et al.: HD (n = 20, range, 0.1%-2.7%; median: 1%), MGUS (n = 42, range, 0-48%; median: 2.05%), MM (n = 82, range, 0-92%; median: 23%); Görgün et al.: NDMM (n = 6, mean = 16.5%), RRM (n = 10, mean = 26.6%), HD (n = 3, mean = 4.7%), Tamura et al. did not report exact results, Ray et al. use MFI scale without units]. As PD-L1 has a uniquely unimodal and homogenous pattern of expression, it is probably more exact to compare its MFI (mean fluorescent intensity) than the percentage of positive cells. Paiva et al. used comparison of MFI as well as gating of only clonal PCs and not total PCs and they, in fact, found no difference in PD-L1 expression between newly diagnosed multiple myeloma (NDMM), MGUS and HD.32 Interestingly, they confirmed a statistically higher expression of PD-L1 on clonal PCs from MRD positive myeloma patients compared with HD as well as higher PD-L1 expression on clonal PCs compared with total PCs in MGUS and MRD positive MM patients.32 In conclusion, it is not currently completely clear that PD-L1 is overexpressed on pathological PCs compared with normal PCs.

Expression of PD-L1 on minor immune cell subsets within myeloma microenvironment

Dendritic cells (DCs) play an important role in the regulation of T cell response against tumors. Three main DC populations are crucial in T cell response: (i) CD303+ plasmacytoid dendritic cells (pDCs), (ii) myeloid dendritic cells (mDCs) CD1c+CD141+ and (iii) mDCs CD1c+CD141- (Collin 2013). Plasmacytoid DCs are increased in the BM of MM patients and contribute to immune dysfunction because of their decreased ability to trigger T cell proliferation (Chauhan 2009). Ray et al. reported that pDCs express high surface levels of PD-L1 (Ray 2015). Also Sponaas et al. found PD-L1 overexpressed on pDCs in 81% of cases. Among myeloid DCs, the PD-L1 expression was much higher on the CD141+ subset that controls CD8+ T cell responses than on the CD141- subset controlling CD4+ T cell responses (Sponaas 2015). All these data suggest that the restoration of normal DC function by PD-1/PD-L1 blockade may contribute to the successful elimination of myeloma tumor cells.

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous, immature myeloid cell population with the ability to suppress innate and adaptive immune responses that promote tumor growth. MDSCs are increased in MM patients and seem to be playing an essential role in disease progression through the orchestration of immune suppression (Malek 2016). Görgün et al. reported that the PD-L1 expression on MDSCs was increased in BM of RRMM patients vs. NDMM as well as MDSCs expressed more PD-L1 than normal antigen presenting cells (APC–CD11b+CD14+HLA-DR-) (Görgün 2015). These findings also support the potential mechanism of action of PD-1/PD-L1 inhibitors via MDSCs.

Expression of PD-1 on T cells

Görgün et al. reported an increased expression of PD-1 on CD4+ T cells from NDMM and relapsed refractory multiple myeloma (RRMM) samples compared with HD and no difference in PD-1 expression on CD8+ T cells.30 Rosenblatt et al. demonstrated that PD-1 expression is upregulated in both CD4+ and CD8+ T cells in patients with advanced MM in comparison with healthy volunteers.33 Finally, Paiva et al. found a similar percentage of PD-1+ T cells between controls and newly diagnosed MM and MGUS, but they confirmed a significant increase in PD-1 expression on both CD4+ and CD8+ T cells in samples from MRD positive MM and relapsing MM patients.32

Expression of PD-1 on NK cells

All published studies have confirmed that PD-1 is absent on normal CD56+CD3- NK cells from HD. However, discordant results are available regarding NK cells from MM patients. Benson et al. and Görgün et al. confirmed a significantly higher expression on NK cells from MM patients compared with HD, whereas Paiva et al. did not find any difference between them.30,32,34

Preclinical data

In vitro experiments

Due to the imperative of HLA restriction, T cells are able to kill tumor cells only after their T cell receptor (TCR) recognizes the peptide–HLA complex on the surface of tumor cells. Because of
this difficulty, the majority of in vitro experiments are performed with autologous PCs and immune cells from MM patients’ BM samples. Görgün et al. separately co-cultured FACS sorted T cells and NK cells with CD138+ MM cells from RMM patients in addition to anti-PD-1, anti-PD-L1, alone or together, and with lenalidomide. They have shown that the blockade of PD-1 and PD-L1 alone, and more significantly, in combination, induces effector cell-mediated anti-myeloma cytotoxicity. They found out that NK cells demonstrated a more pronounced cytotoxicity than T cells, and that lenalidomide further enhances checkpoint blockade-mediated cytotoxicity. Ray et al. co-cultivated freshly isolated CD8+, CD4+ T cells and NK cells from MM patients with autologous pDCs for 5 d in the presence of anti-PD-L1 mAb, after they added MM. S PCs for 3 d. They demonstrated that anti-PD-L1 triggers robust myeloma-specific CD8+ T cell- and NK cell-mediated cytotoxicity, and to a lesser extent also CD4+ T cell-mediated cytotoxicity, evidenced by a decreased number of viable MM.1S cells.

**Murine models**

To date, only three in vivo studies evaluating the efficacy of PD-1/PD-L1 blockade in myeloma mouse models have been performed. Although conducted under completely different conditions, all of them have shown improvement in survival. In the first, PD-L1 blockade was used after autologous stem cell transplantation and administration of whole cell vaccination, demonstrating an improvement in survival from 0% to 40% of myeloma bearing mice. In the second study, the PD-L1 blockade was administered after lymphodepleting irradiation, resulting in the survival of approximately 66% of mice, compare with 0% in the control group. Interestingly, the depletion of either CD4+ or CD8+ T cells completely abrogated the therapeutic efficacy of irradiation plus anti-PD-L1. On the other hand, depletion of NK cells did not significantly affect therapeutic efficacy. In the third study, Paiva et al. used anti-PD-1 mAb alone and also demonstrated significantly superior survival in the treatment cohort.

**Clinical data**

Monoclonal antibodies targeting the PD-1/PD-L1 axis can be logically divided into two groups: (i) those against PD-1 receptors and (ii) those against the ligands (PD-L1/PD-L2). The first group, represented mainly by nivolumab (Bristol-Myers Squibb), pembrolizumab (Merck) and pidilizumab (Medivation/Pfizer), is much further ahead in clinical development with pembrolizumab and nivolumab having reached phase 3 clinical trials, see Table 1. Within the second group, the most promising are durvalumab (Celgene) and atezolizumab (Roche), both anti-PD-L1, which have just entered the early phases of clinical testing, see Table 2.

**Nivolumab**

Nivolumab (OPDIVO) is a fully human IgG4 mAb targeting the PD-1 receptor. A recently published phase I clinical trial evaluating nivolumab monotherapy in patients with relapsed or refractory B-cell or T-cell lymphoma or MM revealed no objective responses in MM patients. From 27 RRMM patients (median of three previous treatments), 63% (17/27) reached stable disease (SD) as a best response (except one patient who reached complete remission, but only after irradiation of the rib because of plasmocytoma). Its safety profile was similar to that seen in solid tumors; immune-mediated adverse events (AEs) occurred in 34% of patients, with pneumonitis being the most frequent (11%).

**Pembrolizumab**

Pembrolizumab (KEYTRUDA) is a highly selective, humanized IgG4 mAb targeting the PD-1 receptor. Preliminary results of the phase 1 study of pembrolizumab and lenalidomide plus low-dose dexamethasone in RRMM patients were presented at the 2016 ASCO (American Society of Clinical Oncology) Annual Meeting. Overall, from 51 patients with a median of four previous treatments, 40 patients were evaluable for efficacy analysis. Overall response rate (ORR) was 50% (20/40) with 13% of very good partial response (VGPR) and 3% of complete response (CR) being achieved. In addition, 48% (19/40) of patients had SD resulting in a disease control rate of 98%. Its safety profile was acceptable with a low rate of immune-mediated AEs and no reported pneumonitis or colitis.

**Pidlizumab**

Pidilizumab (MDV9300, CT-011) is an IgG1 mAb targeting PD-1 receptor. The first preliminary results of phase 1/2 study of pidilizumab plus lenalidomide in RRMM patients were presented at the 2015 ASH (American Society of Hematology) Meeting. From 12 evaluable patients (median of two prior treatments), ORR was 33% (4/12) and another 33% of patients had reached SD. In another clinical trial, pidilizumab is administered in combination with a dendritic cell/myeloma fusion cell vaccination following autologous stem cell transplantation. Already 22 RRMM patients have been enrolled, 27% (6/22) of them reached VGPR, another 27% (6/22) CR, although these results must be interpreted with caution, as it is not clear what kind of treatment is responsible for the outcomes.

Briefly, two randomized phase 3 clinical trials examine the efficacy of pembrolizumab. Pomalidomide + dexamethasone vs. pomalidomide + dexamethasone + pembrolizumab in RRMM patients is compared in KEYNOTE-183, whereas the combination of lenalidomide and dexamethasone with or without pembrolizumab in NDMM is tested in KEYNOTE-185 (30). In another phase 3 trial, the combination of lenalidomide and dexamethasone with or without nivolumab alone or nivolumab with elotuzumab in RRMM patients is tested (Table 1).

**Future directions**

A combination approach seems to be crucial for the successful use of anti-PD-1/PD-L1 mAbs in MM as the results with monotherapy have been unsatisfactory, see Fig. 1. Their most logical partners are IMiDs, the current cornerstone of MM treatment that possess many effects, potentially synergistic, with checkpoint inhibitors such as (i) co-stimulation of T and NK cells, (ii) reduction in Tregs or (iii) direct downregulation...
| Title                                                                 | Experimental arm                                                                 | Active comparator | Condition                                                                 | Estimated enrollment | Identifier           |
|----------------------------------------------------------------------|----------------------------------------------------------------------------------|-------------------|---------------------------------------------------------------------------|----------------------|----------------------|
| Study of pembrolizumab (MK-3475) in combination with dinaciclib*     | Pembrolizumab and Dinaciclib                                                      | x                 | relapsed or refractory multiple myeloma (among others)                    | Active recruitment   | NCT02684617 Phase 1  |
| (MK-37965) in hematologic malignancies (MK-37965–155)(KEYNOTE-155)  |                                                                                  |                   |                                                                           | 138 pat.             |                      |
| A trial of pembrolizumab (MK-3475) in participants with blood cancers| Pembrolizumab                                                                     | x                 | relapsed or refractory multiple myeloma                                   | Active recruitment   | NCT01953692 Phase 1  |
| (MK-3475–013)(KEYNOTE-013)                                          |                                                                                  |                   |                                                                           | 222 pat.             |                      |
| A study of pembrolizumab (MK-3475) in combination with standard of   | Pembrolizumab+Carfilzomib+Dexamethasone                                           | x                 | relapsed or refractory multiple myeloma                                   | Active recruitment   | NCT02096502 Phase 1  |
| care treatments in participants with multiple myeloma (MK-3475–023/KEYNOTE-023) |                                                                                  |                   |                                                                           | 85 pat.              |                      |
| ACP-196* in combination with pembrolizumab, for treatment of hematologic malignancies (KEYNOTE145) | ACP-196 +Pembrolizumab                                                          | x                 | Multiple Myeloma (among others)                                           | Active recruitment   | NCT02362035 Phase 1/2|
| Anti-PD-1 (MK-3475) and IMiD (Pomalidomide) combination immunotherapy in relapsed/refractory multiple myeloma | Pembrolizumab+Pomalidomide +Dexamethasone                                         | x                 | Relapsed or refractory multiple myeloma                                   | Active recruitment   | NCT02289222 Phase 1/2|
| Pembrolizumab (MK-3475) in MM patients with residual disease         | Pembrolizumab                                                                   | x                 | Residual disease of MM                                                     | Active recruitment   | NCT02636010 Phase 2  |
| Phase 2 multi-center study of anti-PD-1 during lymphopenic state after HDT/ASCT for multiple myeloma | HDM → ASCT → Pembrolizumab+Lenalidomide                                           | x                 | Multiple myeloma of any stage                                             | Active recruitment   | NCT02331368 Phase 2  |
| Phase 2 multi-center study of anti-PD-1 during lymphopenic state after HDT/ASCT for multiple myeloma | HDM → ASCT → Lenalidomid+Pembrolizumab                                            | x                 | Multiple myeloma of any stage                                             | Active recruitment   | NCT02331368 Phase 2  |
| Study of pomalidomide and low dose dexamethasone with or without pembrolizumab (MK-3475) in refractory or relapsed and refractory multiple myeloma (rrMM) | Pembrolizumab+Pomalidomide +Dexamethasone                                         | x                 | ≥ 2 lines of treatment (including IMID and Active recruitment)              | 300 pat.             | NCT02576977 Phase 3  |
| Study of lenalidomide and dexamethasone with or without pembrolizumab (MK-3475) in participants with newly diagnosed treatment naive multiple myeloma (MK-3475–185/KEYNOTE-185) | Pembrolizumab+Lenalidomide +Dexamethasone                                        | x                 | Newly diagnosed multiple myeloma, patients ineligible for ASCT            | Active recruitment   | NCT02578663 Phase 3  |
| Pembrolizumab for smouldering multiple myeloma (SMM)                 | Pembrolizumab                                                                   | x                 | Smouldering multiple myeloma                                               | Not yet recruiting   | NCT02603887 Phase NA  |
| Pidilizumab                                                          | Pidilizumab+Lenalidomide                                                         | x                 | Relapsed or refractory multiple myeloma                                   | Active recruitment   | NCT0207959 Phase 1/2 |
| Lenalidomide and pidilizumab in treating patients with relapsed hematologic malignancies after donor stem cell transplant | Pidilizumab +Lenalidomide                                                       | x                 | Relapsed or refractory multiple myeloma                                   | Active recruitment   | NCT01822509 Phase 1  |

Table 1. Ongoing clinical trials with anti-PD-1 mAbs in multiple myeloma.
| Study Description                                                                 | Treatment                                                                 | Phase | Recruitment Status | NCT Number  | Patients |
|----------------------------------------------------------------------------------|----------------------------------------------------------------------------|-------|--------------------|-------------|----------|
| Safety study of nivolumab by itself or in combination with ipilimumab or in combination with lirilumab in patients with lymphoma and multiple myeloma | Nivolumab, Nivolumab+Ipilimumab, Nivolumab+Lirilumab                         | x     | Active recruitment | 315 pat.    | NCT01592370 |
| Study of combined check point inhibition after autologous haematopoietic stem cell transplantation in patients at high risk for post-transplant recurrence (CPIT001) | HDM → ASCT → Nivolumab+Ipilimumab                                          | x     | Not yet recruiting | 42 pat.     | NCT02681302 |
| Study of combinations of nivolumab, elotuzumab, pomalidomide and dexamethasone in multiple myeloma (CheckMate 602) | Nivolumab+Pomalidomide+Dexamethasone, Nivolumab+Pomalidomide+Elotuzumab+Dexamethasone | x     | Active recruitment | 406 pat.    | NCT02726581 |

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1. Dinaciclib—Inhibitor of cyclin-dependent kinases (CDKs)  
2. APC-196—Novel Bruton tyrosine kinase inhibitor  
3. Ipilimumab—Anti-CTLA-4 mAb  
4. Lirilumab—The second-generation anti-KIR mAb  
5. Elotuzumab—Anti-CS1 mAb
| Title                                                                 | Experimental arm | Active comparator | Condition                        | Estimated enrollment | Identifier       |
|----------------------------------------------------------------------|------------------|-------------------|-----------------------------------|----------------------|------------------|
| 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) | Atezolizumab (+ regimens containing) | x                  | Relapsed or refractory multiple myeloma | Active recruitment 214 pat. | NCT0243120 8 Phase 1 |
| Pilot study of anti-programmed death ligand-1 (Anti-PD-L1, Atezolizumab) in asymptomatic myeloma | Atezolizumab     | x                  | Asymptomatic multiple myeloma     | Active recruitment 20 pat. | NCT02784483 Phase 1 |
| Durvalumab (+ regimens containing)                                    |                  |                   |                                   |                      |                  |
| 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 | Durvalumab       | x                  | Relapsed or refractory multiple myeloma | Active recruitment 138 pat. | NCT0261664 0 Phase 1 |
| A study of durvalumab in combination with lenalidomide with and without dexamethasone in subjects with newly diagnosed multiple myeloma | Durvalumab+Lenalidomide+Dexamethasone | x                  | Newly diagnosed multiple myeloma   | Active recruitment 138 pat. | NCT0268582 6 Phase 1 |
| A study to determine the safety and efficacy for the combination of durvalumab and daratumumab in relapsed and refractory multiple myeloma (FUSIONMM-003) | Durvalumab+Daratumumab+Pomalidomide+Dexamethasone | x                  | Relapsed or refractory multiple myeloma | Not yet recruiting 406 pat. | NCT0280745 4 Phase 2 |
of the PD-1/PD-L1 axis.\textsuperscript{40} It was shown that lenalidomide reduces the expression of PD-L1 on myeloma PCs as well as PD-1 on effector cells in BM samples from RRMM patients.\textsuperscript{40} The combination of pembrolizumab with lenalidomide and dexamethasone led to ORRs of 50\% and 48\% of SD in RRMM patients.\textsuperscript{37} Phase 3 clinical trials with lenalidomide + dexamethasone and pomalidomide + dexamethasone have already been launched.

Besides IMiDs here are many potentially synergistic drugs that could be rationally used in combination with PD-1/PD-L1 inhibitors to increase their efficacy. Two recently approved mAbs (daratumumab and elotuzumab) represent a logical option as they kill the tumor cells dominantly via T- and NK cell-mediated cytotoxicity. Therefore, it is apparent that unleashing immune effector cells from the inhibitory effect of PD-1/PD-L1 axis can substantially increase their efficacy.

There is a rationale behind the combination of anti-PD-1/PD-L1 mAbs with anti-CTLA-4 mAbs (ipilimumab). The primary site of action of ipilimumab is during the induction phase of antitumor T cell immunity within lymphoid tissues, whereas anti-PD-1/PD-L1 mAbs primarily act at the effector phase within the tumor microenvironment, suggesting their effects may be additive (Carlino 2016). As it is known from melanoma, the combination of nivolumab and ipilimumab has led to a further improvement in response rate and progression-free survival. However, this combination is associated with an increased rate of immune related toxicities in comparison to monotherapy with each drug (Larkin 2015, Carlino 2016).

Another interesting approach is to combine the inhibition of inhibitory immune checkpoints (anti-PD-1, anti-PD-L1) with the stimulation of stimulatory immune checkpoints (i.e., anti-CD137). Agonist anti-CD137 mAb monotherapy has been examined in myeloma mouse models and its use has

\[\text{Figure 1. Mechanism of action of PD-1/PD-L1 inhibitors and potential synergism with IMiDs.}\]
significantly increased overall survival of treated mice (Murrilo 2008). The combination of anti-CD137 and anti-PD-1 has already been successfully tested in the preclinical models of various solid tumors, and the phase 1/2 clinical trial combining uredulab (stimulating anti-CD137) with nivolumab in advanced solid cancers and B-cell non-Hodgkin lymphomas has been already launched.

Another group of mAbs targeted against killer-cell immunoglobulin-like receptors (KIRs) on the surface of NK cells, aim to augment the NK cell antilymphoma cytoxicity by blocking the inhibitory KIR–ligand relationship (Jelinek 2016). There is an ongoing phase 1 clinical trial combining nivolumab with lirilumab, the second-generation anti-KIR mAb, in lymphoma and myeloma patients.

There has been a recent discovery of a novel mechanism of resistance of MM cells to antilymphoma agents initiated after stimulation of PD-1/PD-L1 pathway. The authors have revealed that the interaction between PD-L1 and PD-1 not only stimulates the PD-1/PD-L1 pathway. The authors have also administered PD-1/PD-L1 mAbs in lymphoma and myeloma patients.

There has been a recent discovery of a novel mechanism of resistance of MM cells to antilymphoma agents initiated after stimulation of PD-1/PD-L1 pathway. The authors have revealed that the interaction between PD-L1 and PD-1 not only inhibits specific cytotoxic T lymphocytes, but also induces drug resistance through the PI3K/AKT signaling pathway. This suggests that there is some merit in the potential combination treatment of PD-1/PD-L1 mAbs with PI3K inhibitors.

Also, another drug interfering in the B-cell receptor pathway, ibrutinib—a covalent inhibitor of Bruton’s tyrosine kinase, could possess synergistic effect with PD-1/PD-L1 inhibitors. As it has been shown on mouse models of B-cell lymphoma, this combination significantly suppresses the tumor growth and warrants further investigation in clinical trials. Ibrutinib is currently being tested in phase 2 studies in combination with novel PI3s as well as with IMiDs in relapsed MM patients.

Radiation therapy (RT) is a procedure that is already well known for its synergism with checkpoint inhibitors. It is a promising candidate for combination treatment based on its capacity to induce not only cancer cell death but also to mobilize immune responses for tumor control. Radiation seems to increase cancer cell killing and DNA release, with a resulting enhancement in dendritic cell-mediated T cell priming and through the so-called “abscopal effect” also localized RT can result in immune-mediated tumor regression in distinct sites out of the irradiated field. To date, only one MM patient treated with anti-PD-1 monotherapy has reached CR, and he was concomitantly irradiated on the rib because of a plasmacytoma lesion. The combination of total marrow irradiation and immune checkpoint blockade, for example, could increase final treatment efficacy.

Finally, immunogenic cell death or immunogenic apoptosis is a form of cell death caused also by some agents routinely used in MM treatment like doxorubicin or bortezomib. This type of apoptosis can induce an effective antitumor immune response through the activation of DCs and the subsequent activation of a specific T cell response, meaning a potential benefit in combination with checkpoint inhibitors.

A key role in this kind of immunotherapy may be played not only by the right combination of drugs, but also by the right timing of treatment initiation. If we consider the fact that PD-L1 expression on malignant cells is higher only in MRD positive patients, then perhaps this is the correct time to start the treatment. Another approach is to administer PD-1/PD-L1 inhibitors during the lymphodepletion period after autologous stem cell transplantation, when the immune system is recovering.

A lot of research should also be conducted to find valid biomarkers that identify patients who would benefit from treatment with PD-1/PD-L1 inhibitors. The expression of PD-L1 on tumor cells as well as the expression of PD-1 on immune effector cells is considered more or less to be a reliable biomarker in some types of solid tumors. There are also FDA approved PD-L1 immunohistochemical assays available which the physician can use to determine which patients will benefit the most from the treatment. It has also been suggested that tumors harboring high levels of somatic mutations may be highly sensitive to immune checkpoint inhibitors; this is yet to be confirmed in MM.

**Conclusion**

MM is a typical prototype of a malignancy with severe defects of humoral as well as cellular immunity. Bone marrow (BM) failure due to the proliferation of PCs, hypogammaglobulinemia, BM cytokine/chemokine microenvironment suppressing local and systemic immunity as well as impaired T cell functions with Th1/Th2 imbalance, expansion of T regulatory cells (Tregs) and others, are the main reasons. Disrupting one of these tumor-supporting pathways by blockade of PD-1/PD-L1 axis seems to be a logical and possibly effective step. Nevertheless, some fundamental conditions have to be met for successful treatment. For example, PD-L1 should be overexpressed on malignant PCs, as it has been described by many authors. This basic postulate is not that clear as the authors differ in their results, and it can be also true that PD-L1 is truly overexpressed only on PCs from MRD positive samples. Analogically, discordant results have been reported about the immune effector cell population responsible for killing PCs. Görög et al. highlighted the higher cytotoxicity of NK cells and Ray et al. considered the most effective CD8+ T cells together with NK cells. On the other hand, Kearn et al. proved that anti-PD-L1 treatment does not work without both CD4+ and CD8+ T cells, but its efficacy is preserved when NK cells are depleted in the mouse model. Another prerequisite for the successful use of checkpoint inhibitors is the presence of the exhausted phenotype of T cells that allows the rescue of their function and restoration of their cytotoxicity. Interestingly, Suen et al. recently discovered that T cells in MM patients have a rather senescent phenotype than an exhausted one, and that this could be the possible reason for sub-optimal response to checkpoint blockade in MM. In reality, monotherapy with this class of drugs has not lead to any clinical response, with SD being the best response in 63% of patients in phase 1 clinical trial with nivolumab in RRMM patients. It is becoming evident that the results of monotherapy treatment with PD-1/PD-L1 inhibitors in MM are unsatisfactory and a combination approach is needed. Immunomodulatory agents that possess many potentially synergistic effects represent the best partner for them. Besides IMiDs there are many other drugs and procedures that warrant further investigation in preclinical as well as clinical trials.

Whether the introduction of immune checkpoint inhibitors will cause the same revolution in myeloma patients as in some...
solid tumors is not yet quite clear. As MM is a genetically and immunologically complex disease, it is possible that targeting only one immune checkpoint pathway will not be sufficient. It is apparent that choosing the right partner and the right timing of the treatment initiation will be of utmost importance.

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No potential conflicts of interest were disclosed.

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Author contributions
TJ wrote the paper and designed the concept. RH proofread the manuscript and took part in coordination of the work.

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