REVIEW ARTICLE

Is immunotherapy here to stay in multiple myeloma?

Paula Rodríguez-Otero,[1] Bruno Paiva,[1] Monika Engelhardt,[2] Felipe Prósper[1] and Jesús F. San Miguel[1]

[1]Clínica Universidad de Navarra, Centro de Investigación Médica Aplicada, Instituto de Investigación Sanitaria de Navarra, Pamplona, Spain and [2]Department of Medicine I, Hematology, Oncology and Stem Cell Transplantation, Medical Center - University of Freiburg, Faculty of Medicine, Germany

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ABSTRACT

Immune escape and impaired immune surveillance have been identified as emerging hallmarks of cancer.[1] Multiple myeloma represents a genuine example of disrupted immune surveillance characterized by: impaired antibody production, deregulation of the T and natural killer cell compartment, disruption of antigen presentation machinery, upregulation of inhibitory surface ligands, and recruitment of immunosuppressive cells. Although the potential value of immunotherapeutic interventions had a clear antecedent in the graft-*versus*-myeloma effect induced by allogeneic stem cell transplant and donor lymphocyte infusions, it is only recently that this field has faced a real revolution. In this review we discuss the current results obtained with immune approaches in patients with multiple myeloma that have placed this disease under the scope of immuno-oncology, bringing new therapeutic opportunities for the treatment of multiple myeloma patients.

Correspondence:
sanmiguel@unav.es

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Introduction

Multiple myeloma (MM) is a malignant disorder of clonal plasma cells (PCs) that represents approximately 1% of cancers and 10% of hematological malignancies.[2] The survival of MM patients has significantly improved over the past two decades, first through the introduction of high-dose therapy followed by autologous stem cell transplantation (ASCT), and more recently due to the use of proteasome inhibitors (PIs) and immunomodulatory drugs (IMiDs) (Figure 1).[3] It is expected that such improvement in patient outcomes will continue in the years to come. Continuous drug development and understanding of the MM biology has led to a landmark achievement, with the approval in 2015 of four new drugs for the treatment of relapse and relapse/refractory MM patients. Two out of these four new drugs are monoclonal antibodies (mABs), elotuzumab and daratumumab, that represent a new passive immune-mediated therapeutic approach for MM patients, and have placed myeloma under the spotlight of the utmost promising field of immuno-oncology (IO). In this article we review the current knowledge of immune disturbance in MM together with the most relevant immunotherapeutic strategies in this disease.

Impaired immune surveillance in MM

The generation of anti-cancer immunity is a complex, multistep process that starts with the release of cancer cell antigens after cell death. Tumor antigens are then processed and presented by antigen-presenting cells (APCs) to effector T cells, that will migrate to the tumor site once they are primed and activated; there, they may recognize the tumor antigens and launch an immune response to eradicate the cancer cells.[4] Unfortunately, tumors display a wide variety of mechanisms that allow them to evade immune control, such as: (a) the production of pro-inflammatory cytokines mediating the suppression of dendritic and T-cell activation and proliferation,[5,6] (b) the disruption of antigen presentation machinery through the down-

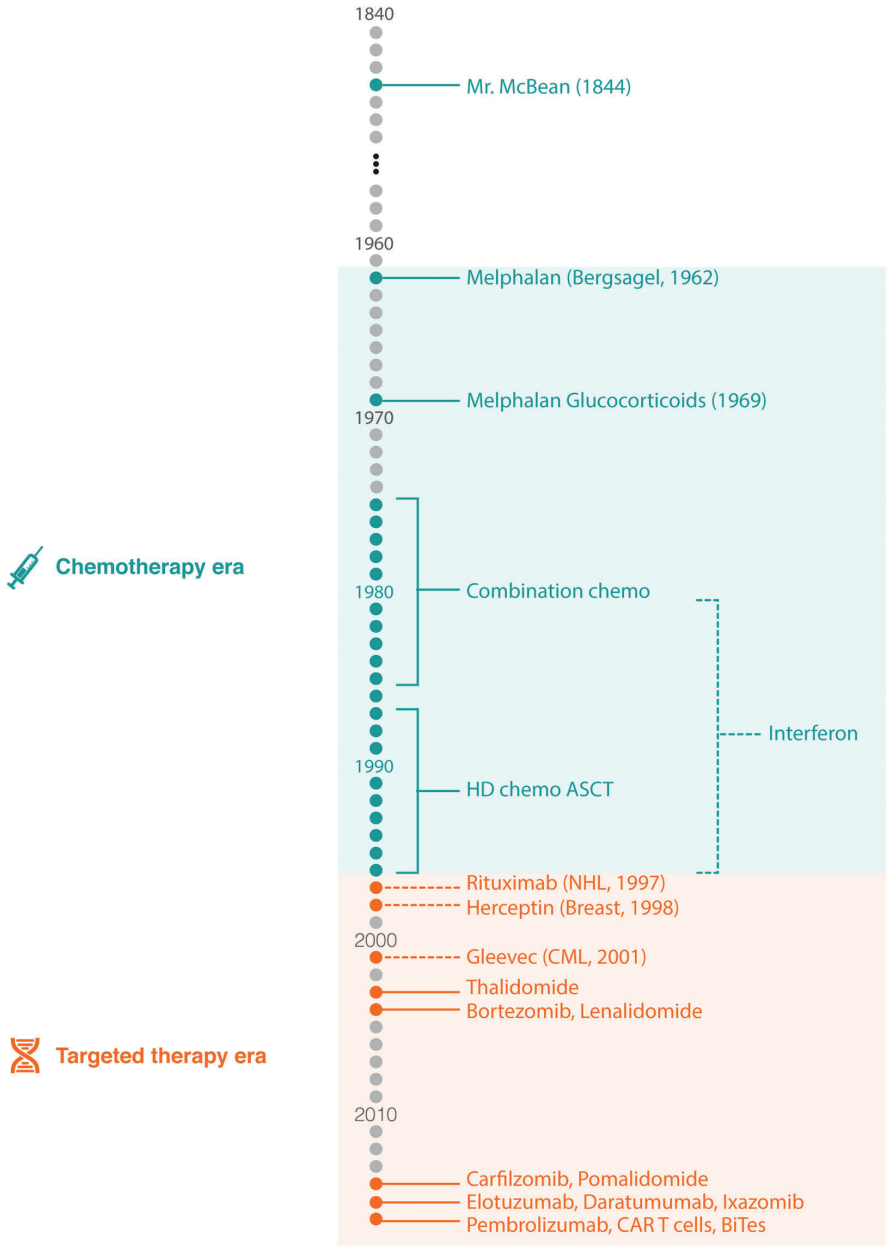

Figure 1. Evolution of the multiple myeloma treatment landscape: multiple myeloma treatment has evolved rapidly over the last years. The first active MM drug developed was melphalan in 1958. From then until 2003 the management of MM patients was mainly focused on the use of high-dose chemotherapy with stem cell rescue. In 2003 the first IMiD was approved (thalidomide), and straightaway bortezomib and lenalidomide were incorporated into the drug repertoire. For ten years these drugs were pivotal in the management of MM treatment, but in the last two years five new drugs have been approved, and immuno-oncology strategies are under development with promising activity. Th: T helper; TGF-β: transforming growth factor-β; VEGF: vascular endothelial growth factor; PGE2: prostaglandin E2; Ab: antibody; HLA: human leucocyte antigen; PD-L1: programmed death-ligand 1; Tregs: regulatory T cells; MDSCs: myeloid-derived suppressor cells; DC: dendritic cell; MDCS: myeloid dendritic cells; CCL2: C-C motif chemokine ligand 2; CXCL12: C-X-C motif chemokine ligand 12.

regulation of human leucocyte antigen (HLA) costimulatory molecules,[7] (c) the upregulation of inhibitory surface ligands that induce T-cell anergy and exhaustion,[8] or (d) the recruitment of immunosuppressive cells.[9,10]

Virtually all these mechanisms of tumor escape have been described in MM (Figure 2), and have been postulated to contribute to disease progression. Firstly, there is a reduction of bone marrow (BM) B-cell precursors that leads to impaired antibody production.[11] Secondly, the T-cell compartment is deregulated due to reduced numbers of $CD4^+$ T cells, altered CD4/CD8 ratio,[12] abnormal T helper (Th)1/Th2 profile in favor of a Th2 immune response,[13] and an increase in the number of regulatory T cells (Tregs).[14,15] Furthermore, MM clonal PCs also express increased levels of inhibitory ligands, such as programmed death-ligand 1 (PD-L1), that inhibits the activation and proliferation of programmed cell death protein 1 (PD-1) positive T cells.[16] Thirdly, MM patients show disruption in antigen presentation, with some studies reporting defects in peripheral blood dendritic cells (DC), such as a reduced number of plasmacytoid dendritic cells (pDCs), myeloid DCs (mDCs) or peripheral blood monocytes, and also a lower expression level of both major histocompatibility complex (MHC) class II (HLA-DR) and costimulatory molecules (CD40, CD80).[17] Fourth, stromal cells produce pro-inflammatory cytokines and other chemokines that recruit immunosuppressive cell populations, such as Tregs and myeloid-derived suppressor cells (MDSCs), thereby creating a permissive microenvironment allowing the tumor to evade immune control.[18-20]

Interestingly, at the same time that a growing body of evidence supported a role for immune dysfunction in the pathogenesis of MM, other examples have also emerged that clearly illustrate the importance of active immune

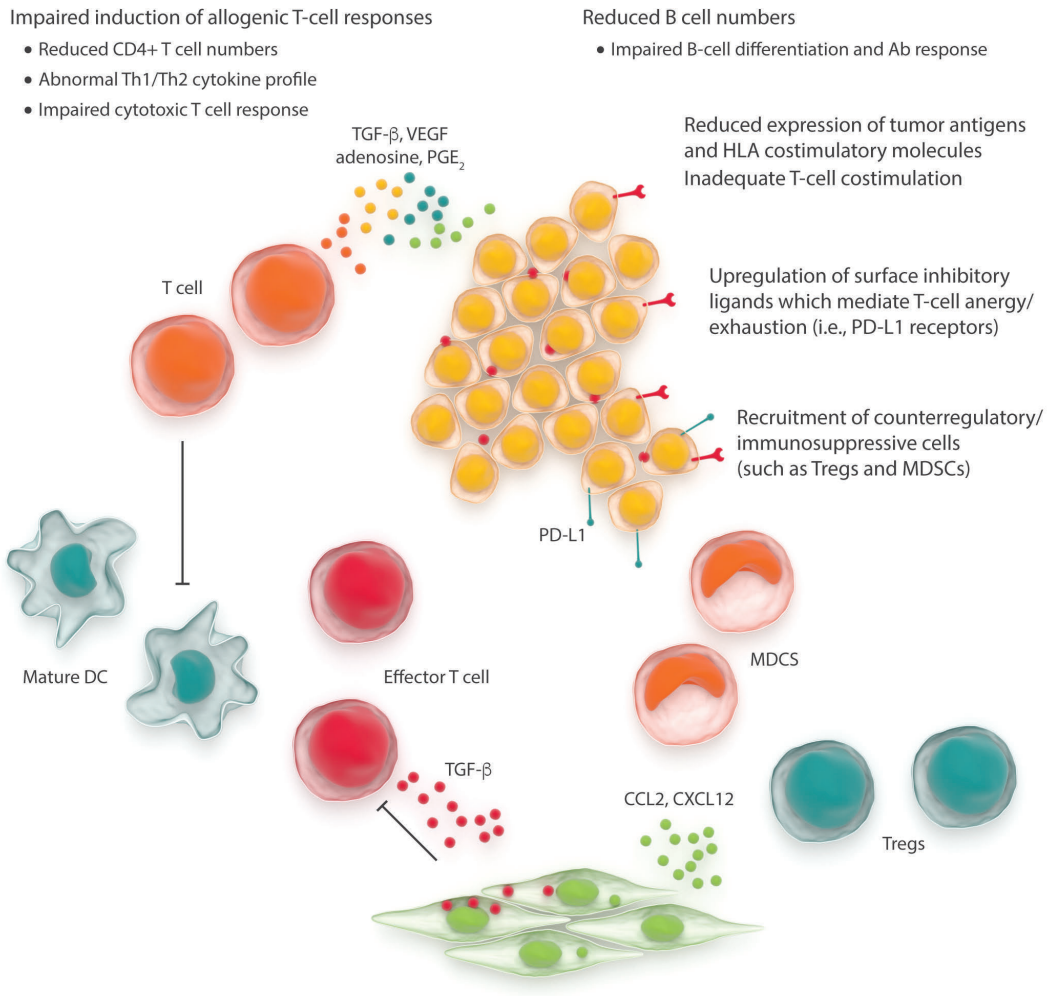

Figure 2. Multiple myeloma is one example of disrupted immunosurveillance and immune evasion. Some evidence underscoring the disturbed immune system in MM are: (a) Impaired induction of allogeneic T-cell responses due to a decrease in the number of $CD4^+$ T cells, and an abnormal Th1/TH2 cytokine profile; (b) reduction in the B-cell compartment with altered B-cell differentiation and antibody response; (c) decrease in the expression of tumor antigens and HLA costimulatory molecules leading to inadequate T-cell costimulation; (d) upregulation of inhibitory ligands such as PD-L1 which mediate anergy and T-cell exhaustion; (e) recruitment of immunosuppressive cell populations like MDSCs or Tregs. IMiDs: immunomodulatory drugs; inh: inhibitor.

surveillance in this disease. In the QUIREDEX trial, early treatment with lenalidomide and low-dose dexamethasone was compared to abstention in high-risk smoldering multiple myeloma (SMM) patients. It was observed that high-risk SMM patients already presented an impaired immune system at the moment of diagnosis as compared to healthy individuals of the same age, with a decreased expression of activation, Th1 and proliferation-related markers in immune cells. After nine induction cycles with lenalidomide and low-dose dexamethasone, the expression of these markers was restored and a shift in T-cell and natural killer (NK) cell phenotype was induced, with an increase in central memory T cells (CMTs), effector memory T cells (EMTs), the induction of activation markers and an increase in proliferating $CD4^+$ and $CD8^+$ cells.[21] Another example is the graft-*versus*-myeloma effect of allogeneic stem cell transplant (SCT), which has been highlighted recently by Ladetto *et al.* upon comparing outcomes between minimal residual disease (MRD) positive and negative patients after tandem auto-allo SCT using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR). With a median follow-up of 12 years, 73% of the MRD-negative cases remained relapse-free; such promising results in MRD-negative patients have never been observed outside of the allogeneic setting.[22] Furthermore, by using 8-color flow cytometry to simultaneously assess MRD and characterize patients' immune profile, we were able to show that a few MRD positive cases - those showing a strong recovery of the normal B-cell lymphopoiesis - have similar outcomes to those of MRD negative patients.[23] This suggests that despite MRD positivity, the intact immune surveillance was profitable in these patients. Finally, we have observed that MM patients attaining long-term survival (i.e., progression-free survival [PFS] for more than 10 years) showed a unique immune profile with a higher number of effector cells (T cells, NK cells, DCs, normal PCs), and a lower number of Tregs, underlying the importance of an active immune system to control disease evolution.[24]

Four major targets of immunotherapy in multiple myeloma

The first evidence supporting a role for immunotherapy in MM comes from the graft-*versus*-myeloma effect induced by allogeneic SCT (allo-SCT) and donor lymphocyte infusions, that may cure some MM patients.[25-28] However, the substantial toxicity of this procedure, along with the occurrence of relapse of the disease after the transplant, has hampered its extensive use. As for pharmacological approaches, interferon was the first drug used to stimulate the immune system, but its efficacy was only modest, and thus it is not currently considered as part of the myeloma treatment armamentarium; despite this, recent publications underlining its potential role in combination with other IO drugs, such as checkpoint inhibitors, may reactivate its use in the future.[29,30] Subsequently, we had the opportunity to experience the anti-myeloma efficacy of a new class of compounds, the IMiDs (thalidomide, lenalidomide and pomalidomide) that represent one of the key backbones in the treatment of MM. Herein, we review four novel and most promising approaches that are currently under investigation to enhance the immune system against MM cells (Figure 3). These are: 1. direct targeting of surface tumor antigens with monoclonal antibodies, 2. boosting immune effector using adoptive cell therapy, 3. improving immunity against tumors with vaccines, and 4. overcoming immune suppression with checkpoint blockade.

Direct targeting of surface tumor antigens using monoclonal antibodies (mAb)

Monoclonal antibodies exert their cytotoxic function through different mechanisms: antibody-dependent cellular cytotoxicity (ADCC) through the engagement of immune effector cells, complement activation, antibody-dependent phagocytosis, and direct effect on target cells acting through different signaling pathways. Although these mechanisms are postulated based on *in vitro* studies, their relative contribution to the clinical responses of mAb therapy is difficult to determine.[31]

While mAb therapy is already a standard of care in the treatment of some hematological malignancies, such as B-cell lymphoproliferative disorders,[32] it was not until recently that this therapeutic approach was made available for MM patients. The development of effective mAb therapies in MM has probably been hindered due to both the lack of knowledge about specific PC targets (e.g., SLAMF7 or BCMA), and the concern that other highly expressed molecules on PCs were also relatively abundant in other hematopoietic cells, which would result in significant off-target effects. Nowadays, there are two mAbs, elotuzumab and daratumumab, approved for the treatment of MM. Elotuzumab is an IgG1κ mAb with specificity against SLAMF7, an antigen expressed on both normal and malignant PCs as well as NK and T cells.[33] Elotuzumab used as a single agent does not induce objective responses in MM, but in combination with lenalidomide plus dexamethasone (Rd) in a phase II trial showed high activity with an overall response rate (ORR) of up to 92%.[34] These results were the basis for the randomized phase III Eloquent-2 trial comparing elotuzumab plus Rd *versus* Rd in relapsed/refractory MM (RRMM) patients. In this trial, the experimental arm showed a significant superiority in terms of ORR (79% *vs.* 66%), PFS (19.4 *vs.* 14.9 months, [HR 0.73, 95%CI 0.60-0.89]; *P*=0.0014) and overall survival (OS) (43.7 *vs.* 39.6 months, respectively), and a delay of 12 months in the time to next treatment (TNT) (HR=0.62, 95%CI 0.50-0,77).[35]

Regarding anti-CD38 mAbs, three different compounds, daratumumab, isatuximab and MOR22 (MORO3O87), are currently being investigated.

Daratumumab has shown clear activity as a single agent, as it has recently been updated in a pooled analysis of 148 patients with RRMM having received more than two prior lines of therapy. Daratumumab was given at the standard dose of 16mg/kg.[36-38] It should be noted that 86.5% of the patients were double refractory (to PI and IMiD). The ORR was 31.1%, including thirteen very good partial responses (VGPRs), four complete responses (CRs), and three stringent complete responses (sCRs). The median duration of response was 7.6 months. Median PFS and OS were 4.0 months and 20.1 months, respectively. The toxicity profile was very acceptable. No immunogenicity was observed, and the most commonly reported adverse events were infusion-related reactions that occurred predominantly during the first full infusion. Both the FDA and EMA have already approved daratumumab for this indication.

The efficacy markedly increased upon combining daratumumab with Rd, with an ORR of 81%.[39] These positive results were the basis of a large randomized phase III POLLUX trial that compared the triple combination of daratumumab plus Rd (DRd) with the standard two drug combination, Rd, in relapsed patients not refractory to lenalidomide.[40] The most recently presented results showed a highly significant superiority for the experimental arm in terms of ORR (93% *vs.* 76%), CR rate (43% *vs.* 19%), TNT (not reached (NR) *vs.* 18.4 months), and PFS (NR *vs.* 18.4 months, HR 0.37 [0.27-0.52], *P*<0.0001), with an unprecedented estimated median PFS for the triplet DRd arm of 44 months in relapsed MM. Similar positive results have also been presented in the phase III randomized CASTOR trial that compared daratumumab, bortezomib plus dexamethasone (DVd) with bortezomib plus dexamethasone (Vd) in relapsed patients not refractory to bortezomib.[41] Again, the triplet combination of DVd was superior to the control arm in terms of ORR (83% *vs.* 63%), CR rate (20% *vs.* 9%), median TNT (NR *vs.* 7.3 months, HR: 0.30 (95% CI, 0.21-0.43); *P*<0.0001) and also for PFS, as the median PFS for the triplet arm was NR *vs.* 7.2 months in the Vd arm (HR: 0.39 (95% CI, 0.28-0.53); *P*<0.0001). Other combinations with carfilzomib plus dexamethasone and also pomalidomide plus dexamethasone are under investigation in a phase I trial (*clinicaltrials.gov Identifier*:01998971). Preliminary results for the daratumumab plus pomalidomide plus dexamethasone combination have shown an ORR of 71% with a CR of 9% in a RRMM population.[42]

As far as isatuximab is concerned, a phase I study identified 10 mg/kg as the optimal dose with a response rate of 29% used as a single agent; infusion reactions were mainly grade 1/2 and only during the first doses.[43] The efficacy of isatuximab in combination with Rd was confirmed in a small pilot study with a response rate of 58%, including 6% sCR and 23% VGPR, in patients that were mostly (84%) refractory to lenalidomide.[44] In general, the safety profile of these mAbs, both elotuzumab and anti-CD38 mAbs, is manageable, and infusion-related reactions (IRRs) are the more frequent adverse events, present in

Figure 3. There are four major targets for cancer immunotherapy. 1. Direct target of surface tumor antigens with monoclonal antibodies; 2. Boost immune effector using adoptive cell therapy; 3. Improve immunity against tumors with vaccines; 4. Overcome immune suppression with checkpoint blockade. Chemo: chemotherapy; HD: high-dose; ASCT: autologous stem cell transplantation; CAR: chimeric antigen receptors; BiTEs: bispecific T-cell engagers; CML: chronic myelogenous leukemia; NHL: non-Hodgkin lymphoma.

around 10% of patients for elotuzumab and 48% of patients for daratumumab. IRRs are more frequent during the first infusion, and most are grade 1-2, with virtually no patients having to be discontinued. Two other issues that need to be stressed regarding mAbs treatment are the interference in both the evaluation of response and in the blood typing. The first problem applies to all mAbs, considering that they are immunoglobulins (most are IgGκ) that could be detected in both the serum protein electrophoresis (SPEP) and the immunofixation test. The second problem is limited to anti-CD38 antibodies (Abs), since CD38 antigen is also expressed on the surface of red blood cells, thus interfering in blood typing because of a false positive indirect Coombs test.

There is further development in the field of mAbs with the use of bispecific antibodies, such as bispecific T-cell engagers (BiTEs). These drugs combine the specificities of two antibodies; one involves the engagement and activation of T cells *via* CD3, and the other recognizes the cancer antigen. This class of drugs may overcome the inhibition of an immunosuppressive microenvironment because they activate and bind the effector T cell to the tumor cell, and thereby lead to an increased lytic potential of autologous effector T cells.[45] The first BiTE to be generated against myeloma cells was developed by combining single-chain variable fragments (ScFvs) of a mAb that binds normal and malignant PCs (Wue-1).[46] Other BiTEs are under development using other antigens, such as B-cell maturation antigen (BCMA).[47] Antibodies can also be conjugated with cytotoxic molecules, such as monomethyl auristatin E (e.g., ABBV-838), or radioactive particles.[48] Both technologies are also being explored in MM, both in preclinical and clinical studies (*clinicaltrials.gov Identifier*:02462525).

Boosting immune effectors through adoptive cell therapy

A second strategy to improve and/or increase immunity against cancer would be the use of adoptive cell therapy (ACT) either with tumor-infiltrating lymphocytes (TILs), NK cells,[49-51] or engineered T cells.[52] Natural TILs are typically anergic *in vivo* by the expression of immunosuppressive molecules, such as PD-1, LAG-3 or CTLA-4. Removing T cells from the tumor immunosuppressive environment enables their activation and expansion.[53,54] The reinfusion of these cells after *ex vivo* expansion can trigger the eradication of the tumor.[55,56] The emergence of neo-antigens is an important factor contributing to the

efficacy of TILs, which explains why this approach has mainly been used in solid tumors (e.g., melanoma) rather than in hematological malignancies.[57,58] Clinical experience with TILs in MM is scanty, however, the work from Borrello *et al*. with marrow-infiltrating lymphocytes (MILs) is encouraging, with twenty-three patients treated with MILs in the setting of ASCT with evidence of anti-myeloma immunity, effective trafficking of the MILs to the BM, persistence over time, and correlation between clinical response and myeloma-specific immunity,[55] demonstrating the feasibility of, and interest in, the approach.

Progress in gene engineering technologies has simplified the generation of specific antitumor T cells, overcoming many of the practical barriers that have limited wide dissemination of ACT using TIL cells.[59,60] Theoretically, gene engineering may well be capable of targeting virtually any cancer histology. Genetically redirecting a T-cell's specificity toward a patient's cancer cell can be accomplished in two ways. In one approach a cloned T-cell receptor (TCR) conferring tumor recognition is inserted into circulating lymphocytes. Similarly to the endogenous TCR, genetically inserted TCRs recognized tumor antigens within the groove of a specific MHC molecule. In a second approach, an alternative way to provide specificity to transduced T cells and overcome some of the limitations of TCR engineered T cells, is with the use of chimeric antigen receptors (CARs).[52,61] CARs are engineered fusion proteins that contain an extracellular antigen-binding domain composed of a ScFv derived from an Ab, that confers recognition to a tumor-associated antigen, linked in tandem to intracellular signaling motifs capable of T-cell activation, such as CD3z, or costimulatory molecules, like CD28 or CD137.[62] By means of retroviral or lentiviral transduction, or by electroporation transfer, patient's T cells express the CAR.

Both CAR and TCR T cells have some advantages and limitations. CAR T cells are not restricted by the human leukocyte antigen of the patient, but selecting the appropriate antigen is critical to prevent off-target toxicity. Many potential targets used for CAR T-cell immunotherapy have a broad expression across normal cells and tissues, therefore requiring careful evaluation.[63] Another advantage of CAR T-cell therapy is the possibility to insert other genes encoding molecules involved in costimulation, survival, proliferation or inflammation, allowing the T cell to avoid inhibitory mechanisms displayed by the tumor.[64,65] Differently from CAR T cells, TCR-engineered T cells recognize antigens presented by specific HLA molecules. Accordingly, only a limited number of individuals presenting such HLA molecules are eligible for this treatment option.[66] However, TCR, but not CAR, T cells can recognize intracellular proteins, providing a broader array of potential therapeutic targets.[52]

There are other considerations concerning both strategies that need to be stressed. Potent antitumor effect without off-target damage can occur if the target is only expressed in the tumor cells, such as NY-ESO TCR T cells.[67] However, if the T cells are modified with a receptor that recognizes the antigen both in malignant and non-malignant cells, like anti-CD19 CAR T cells, normal cells will be equally attacked resulting in the off-target effects seen with these therapies that are actually limiting its availability.[68] Moreover, there is risk of a massive release of pro-inflammatory cytokines produced by hyperactive CAR T cells, resulting in cytokine release syndrome (CRS), characterized by fever, hypotension or renal failure.[69]

CAR T cells with CD19 specificity have been used for the treatment of lymphoid malignancies, with impressive clinical results mainly in refractory B-cell acute lymphocytic leukemia (ALL) patients.[70,71] The same anti-CD19 CAR T-cell model has been used in one refractory myeloma patient with intriguing results (CR after ASCT and CAR T-cell infusion with a response duration of longer than 12 months), despite the fact that clonal PCs did not express CD19; 99.95% were negative for this antigen.[72] Other targets, such as BCMA, a surface antigen expressed on normal and malignant PCs, have also been used for CAR[73,74] development as well as for BiTEs in RRMM patients. In the first-in-human trial using CAR T cells with BCMA specificity, twelve patients were treated, with four out of twelve responders, including one patient achieving a sCR (two PR, one VGPR, one sCR).[75] Of note, the patient that achieved sCR experienced a cytokine release syndrome including fever, tachycardia, hypotension, elevated liver enzymes, and elevated creatine kinase, all of which resolved in two weeks or less.

TCR-engineered T cells with NY-ESO-1 and LAGE-1 specificity have also been tested in MM patients. In a phase I/II trial, twenty patients with active MM (six patients at diagnosis and fourteen at relapse) were treated with NY-ESO-1- and LAGE-1-specific TCR-engineered T cells. Cells were infused on day +2 after ASCT conditioned with high-dose melphalan and followed by lenalidomide maintenance. Fourteen out of twenty patients achieved at least near complete response after the planned treatment, although it is difficult to dissect the specific contribution of the TCR-engineered T cells from ASCT or maintenance treatment. Interestingly, affinity-enhanced T cells showed extended persistence (more than two years in some patients), and MM progression correlated either with a loss of persisting TCR T cells or the appearance of a negative subclone.

Improving antigen-specific immunity using vaccines

Although surface antigens can serve as targets for Ab-based therapeutics, most cancer-associated or cancer-specific antigens are derived from intracellular proteins. Thus, another strategy to enhance anticancer immunity would be the use of vaccines to improve the immune response against cancer. There are different vaccination strategies.[76] It should be noted that the choice of the antigen, vaccine formulation, delivery system, adjuvant, immunomodulation, treatment schedule and treatment setting can all modify the quality and strength of the T-cell response vaccines are expected to induce.[76] Hematological malignancies are an opportunity for vaccination development given the relatively high availability of cellular antigens, the possibility of using whole tumor cell lysates to charge APCs as well as the use of a post-transplant setting as a window of opportunity for vaccination, based on the "resetting" of immune relations seen after this procedure.

Several groups have investigated the value of cancer vaccines in MM.[77,78] Two separate vaccination approaches have been developed, one using peptide-based and the other using dendritic cell fusion vaccines. Several trials are ongoing which are evaluating the efficacy of peptide vaccination using different antigens, alone or in combination, such as NY-ESO-1, MAGE-AE, WT-1, and XBP-1, which are broadly expressed in MM cells from selected

patients.[79-83] Globally, vaccination is able to induce immune response, but so far its clinical efficacy remains modest. On the other hand, dendritic cell fusion vaccines exploit the ability of dendritic cells to present several tumor antigens to the host immune effector cells. This strategy has also been evaluated in phase I-II trials in two single institutions. In the first phase I trial, patients with active MM with a median of four prior lines of treatment were treated with the DC/MM fusion vaccine. Eleven out of sixteen evaluable patients achieved stabilization of the disease after vaccination. Vaccination was well tolerated, and was capable of inducing immune responses against myeloma cells.[84] A second phase II trial using the same DC/MM cell fusion vaccine approach in the context of an ASCT, showed a CR/VGPR rate of 78% early after ASCT, and 24% of patients improved their response from PR to CR/near complete remission (nCR) after vaccination at more than three months after ASCT, thus suggesting a vaccine-mediated effect on residual disease.[85] A phase III trial is ongoing to confirm these results (CTN 1401), also in the post-ASCT setting. An innovative vaccination approach has recently been reported using intravenously administered ribonucleic acid (RNA)-lipoplexes (RNA-LPX) to enhance DCs. RNA-LPX encoding viral or mutant neo-antigens or endogenous self-antigens induce strong effector and memory T-cell responses, and mediate potent IFNα-dependent rejection of progressive tumors. A phase I dose-escalation trial is ongoing to further evaluate this strategy.[86]

Overcoming inhibitory immune suppression with checkpoint inhibitors

The limited clinical benefit of vaccines could be related, at least in part, to the inhibition of tumor-specific effector cells through the expression of checkpoint receptors. The activation of T cells includes a two-step process: 1) interaction between the TCR and the antigen presented by MHC molecules, and 2) costimulatory signals to enhance T-cell activation. In the absence of this signal, T cells fail to respond and become inactivated. Under normal physiological conditions, immune checkpoints are essential for the maintenance of self-tolerance and to protect tissues from the potential damage of an exacerbated T-cell response. There are two types of checkpoint receptors: inhibitory receptors, like CTLA-4, PD-1, TIM-3, or LAG-3, and stimulatory receptors, such as CD28, CD137, or OX40, among others.[9,87] T-cell responses can be modulated either using agonist antibodies directed to stimulatory receptors that amplify the immune response, or using antibodies to block inhibitory receptors and release the brakes of immune cells.[9]

Different checkpoint drugs are under development, targeting both activating receptors and also inhibitory receptors. The latter are more advanced in their clinical development, and can be divided into three groups according to their respective targets; CTLA-4, PD-1, and PD-L1 inhibitors.[88] The first checkpoint receptor explored for treatment intervention was the cytotoxic T-Lymphocyte antigen 4 (CTLA-4), and ipilimumab was the first drug administered to target this checkpoint. CTLA-4 is an inhibitory receptor that regulates T cells during the initial activation steps of the immune response; thus it is expressed in the surface of activated and regulatory T cells. When CTLA-4 binds to its ligands (CD80 and CD86), the result is T-cell inhibition interfering with interleukin-2 (IL-2) secretion and interleukin-2 receptor (IL-2R) expression.[9,88,89]

The second group of checkpoint inhibitors are the programmed death receptor-1 (PD-1) inhibitors, nivolumab, pembrolizumab, or pidilizumab. PD-1 is, like CTLA-4, an inhibitory receptor that is expressed on the surface of activated T cells to limit their activity at later stages of immune responses. PD-L1 and PD-L2 are the two ligands of PD-1 and they are expressed on the surface of APC and tumor cells. The binding of PD-1 to its ligand induces the inhibition of T cells.[9,88] Cancer cells upregulate PD-L1 to take advantage of the PD-1 pathway and create an immunosuppressive milieu. Such upregulation of PD-L1 expression has been described in different cancer types, such as melanoma, non-small cell lung cancer, and also in MM, and this expression has been typically linked to poor clinical outcomes.[90-92] Furthermore, TILs have also been shown to express significantly higher levels of PD-1, which could be induced by increased levels of proinflammatory cytokines, such as IFNγ in the tumor microenvironment. Altogether, cancer cells evade immune responses through higher PD-L1 expression on tumors along with higher PD-1 expression on TILs.[54,93-96] In MM, PD-L1 is expressed in clonal PCs across all disease stages, but this expression is significantly higher at relapse and in MRD positive patients. Similarly, expression of PD-1 on T cells was also increased in the BM of patients at relapse and with MRD.[16]

The first results of PD-1 blockade in hematological malignancies were obtained in a phase I trial with nivolumab.[97] ORR reported in diffuse large B-cell lymphoma and follicular lymphoma were 36% and 40%, respectively. In Hodgkin lymphoma, ORR reached 87% in a heavily pretreated and refractory population with 17% CRs. PD-1 blockade in MM patients alone has not induced objective responses, and only 67% of patients with stabilization of the disease were noted.[97] Experimental data has shown that lenalidomide reduces PD-L1 and PD-1 expression on MM cells, T cells, and MDSCs, respectively. Moreover, a clear synergism between lenalidomide and anti-PD-1 and anti-PD-L1 was also observed.[98] Therefore, based on this preclinical data showing a synergistic effect between PD-1 blockade and IMiDs, combination strategies with PD-L1 blockade and lenalidomide or pomalidomide are under evaluation. Data on two phase I trials combining pembrolizumab and IMiDs has been recently reported. The phase I KEYNOTE-023 trial is evaluating pembrolizumab in combination with Rd for RRMM patients that received more than two prior lines of therapy, including both IMiDs and a PI. A total of sixty-two patients were included with a median of four prior lines of therapy, and 76% of the patients were refractory to lenalidomide, with 50% being double, triple or quadruple refractory. Efficacy data, recently updated at the 2016 American Society of Clinical Oncology (ASCO) meeting, showed a 50% rate of ORR in the overall population (n=40) and 36% in lenalidomide-refractory patients. Overall the combination was well tolerated, and the adverse effects were consistent with those observed for pembrolizumab and lenalidomide in their respectively approved indications. It should be noted that immune-related adverse events, such as pneumonitis, hepatitis or colitis, which are typically described with these types of therapies, have not been observed so far in this trial; nevertheless longer follow up is still needed in order to clearly

assess the frequency and severity of these adverse events.[99]

Another currently ongoing phase I/II trial is evaluating the efficacy and safety of pembrolizumab in combination with pomalidomide and low-dose dexamethasone in a similar patient population, that of RRMM having received more than two prior lines of therapy, including an IMiD and a PI. A total of thirty-eight patients have been included, with 89% of them being refractory to lenalidomide and 70% double refractory. ORR in the total population was 66% and 65% in lenalidomide-refractory patients, and a median PFS of 14 months was reported at last follow up. The safety profile was acceptable, with 38% of patients suffering immune-related adverse events (IRAEs), and 14% of patients experiencing pneumonitis.[100] Overall, both combinations are well tolerated and show promising preliminary efficacy in the heavily pretreated RRMM population, but further studies with a larger series of patients and longer follow up are needed to confirm these results.

Future perspectives

The clinical success of checkpoint inhibition, particularly in solid tumors, has reignited the interest in immunotherapy against cancer, and this field is now moving forward very rapidly. Nevertheless, there are still many open questions.

1. It is important to define the target populations that will benefit most from specific immunotherapeutic strategies. So far these therapies have been mainly explored in the relapse setting, but a higher efficacy, specially for checkpoint inhibitors, would probably be expected at stages when a better preserved immune system exists. For this reason combinatorial strategies, including immunotherapeutic agents, should be evaluated in other patient populations, such as in newly diagnosed patients, high-risk SMM and high-risk myeloma patients in early relapse after transplantation, or after consolidation treatment in patients who didn't achieve CR or VGPR. Even more interesting would be to test their efficacy at the time of MRD persistence, maintenance treatment or biochemical relapse in order to improve immune surveillance against residual myeloma cells. Accordingly, specific clinical trials would be welcome in these patient cohorts.

2. We need new biomarkers to predict response to certain treatments, such as the expression of PD-1 or PD-L1, the mutational load or microsatellite instability in the case of checkpoint inhibitors.

3. There is also a need for accurate immune profiling at baseline, to try to identify ideal candidates for therapy, and immune monitoring to identify those patients who would benefit the most.

4. Do we need novel immune-related response criteria or new clinical endpoints, such as TNT? The answer is probably "yes", since some patients may not achieve a CR, or can experience an indolent relapse that may remain under the control of the immune system for longer periods than that currently observed upon using approved anti-myeloma agents.

6. Can we be successful with one immune approach or do we need combination therapies? Immune therapeutic strategies targeting only one pathway are often ineffective or short-lived, and scientific rationale supports the hypothesis that the combination of two or three approaches may enhance the clinical activity of immunotherapy strategies. Then, the challenge will be how to combine or sequence multiple drugs. Combinations will probably need to be based upon the specificity of their mechanism of action and disease stage.

7. Eventually, we have to elucidate how much can we rely on pre-clinical data to guide the design of clinical trials, and how can we possibly improve on this most effectively, with multicenter and multinational activities.

Although there are numerous open questions to address and solve for immunotherapy approaches in MM, this is a fascinating time for myeloma therapy, and current data (supporting the old "immune" experience with donor lymphocyte infusions) already indicate that immunotherapy will be a backbone that might revolutionize treatment, and hopefully improve patient outcomes further.

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