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Immunotherapy strategies for multiple myeloma: the present and the future

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Abstract

Growing knowledge of the complexities of the immune system have led to a better understanding of how it can be harnessed for the purpose of anticancer therapy. Moreover, recent success with immunotherapies for solid tumors, combined with novel therapeutic strategies against myeloma, heighten excitement at the prospect of improving clinical outcomes for myeloma by improving antitumor immunity. Increased understanding of myeloma tumor-associated antigens, availability of more potent vaccines, expanded immune-modulating therapies, development of agents that block immune-suppressive pathways, increased sophistication of adoptive cell therapy techniques and capitalization upon standard autologous transplant are all important standalone or combination strategies that might ultimately improve prognosis of patients with multiple myeloma.

Keywords

cellular therapy; immunotherapy; monoclonal antibody; multiple myeloma; tumor-associated antigen; vaccine

Significant therapeutic strides have taken place in multiple myeloma (MM) with the introduction of novel agents such as lenalidomide and bortezomib, among others [1]. Unfortunately, disease resistance to these agents develops eventually, and patients inevitably face progression accompanied, in general, by a relatively short life expectancy [2]. Numerous immunotherapeutic strategies against MM have attempted to reduce tumor burden and prolong life [3], yet all these efforts have not translated into an obvious clinical benefit and remain investigational at the present time [4]. Emerging immunotherapeutic approaches, such as the recent US FDA approval of the first cancer treatment vaccine, sipuleucel-T for prostate cancer, and the first inhibitor of a negative regulator of immune response,

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ipilimumab for melanoma, are paving the way for similar therapies of other diseases. Although promising, these newer immunotherapy strategies have only achieved limited survival advantages despite tremendous developmental effort and cost. Growing knowledge of the complexities of the immune system provides a better understanding of how it can be harnessed for practical application, such as anticancer therapy. Here, we provide an overview of emerging therapeutic strategies and cutting-edge technologies designed to exploit immune responses against MM.

Evidence of immune control in MM

Allogeneic hematopoietic cell transplant

Allogeneic hematopoietic cell transplant (allo-HCT) remains investigational for the treatment of MM primarily due to significant morbidity and mortality from graft-versus-host disease (GVHD) [5]. However, allo-HCT is a form of donor T-cell-mediated adoptive immunotherapy, with a potential for prolonged disease-free survival in patients with MM. Better understanding of the graft-versus-malignancy effect mediated by donor T cells has revolutionized the field by shifting the focus from myeloablative preparative regimens to more tolerable, reduced-intensity conditioning (RIC) regimens [6]. Reduced toxicity associated with RIC regimens has expanded the pool of patients eligible for hematopoietic cell allografting including those of advanced age or with associated comorbidities. Badros *et al*. described encouraging responses in 31 subjects with relapsed MM who received a RIC allo-HCT [7]. In their series, 19 (61%) achieved a complete (CR) or near-complete response at the expense of a 2-year transplant-related mortality (TRM) of 29% [7]. This encouraging CR rate was noteworthy considering that 17 of 31 cases (55%) had received at least two autologous HCT (auto-HCT) and 17 (55%) had progressive disease (PD) at the time of allo-HCT [7]. This would suggest that a graft-versus-malignancy effect mediated by allogeneic T cells is capable of overcoming drug resistance and inducing disease responses, even when refractory to previous high-dose chemotherapy. Similarly, high responses and encouraging survival is seen when RIC allo-HCT is offered earlier in the course of the disease [8]. In our series, 22 subjects with chemosensitive disease (CR: 45%; very good partial response: 55%) underwent a RIC regimen of fludarabine plus melphalan (n = 13; 59%) with or without bortezomib (n = 9; 41%), then received granulocyte-colony stimulating factor mobilized peripheral blood stem cells from a HLA-matched-related (n = 9; 41%) or matched-unrelated (n = 13; 59%) donor [8]. All patients engrafted at a median of 15 (11–19) days [8]. Results are promising as achievement of stringent CR was observed in 68% of cases (from 27% at the time of allo-HCT); the 2-year cumulative incidence of relapse was 8.3% (95%CI: 0.4%–32.4%); and the 2-year progression-free survival (PFS) and overall survival (OS) were 74.8% and 77.5%, respectively. Four patients died from GVHD resulting in a 2-year cumulative incidence of TRM of 16.9% [8]. These data suggest that allo-HCT is an effective strategy to induce deep and durable responses, but at the expense of significant TRM resulting from GVHD. A recent meta-analysis that compared tandem auto-HCT versus a sequential strategy of auto-HCT followed by allo-HCT failed to show improvement in OS (hazard ratio: 0.80; 95% CI: 0.48–1.32; p = 0.39) despite higher CR rates achieved in the RIC allo-HCT arm (risk ratio: 1.65; 95% CI: 1.25–2.19; p = 0.0005) [9]. It is conceivable that the higher TRM (relative risk: 3.55; 95% CI: 2.17–5.80; p < 0.00001) counterbalanced

the higher CR rates observed following RIC allo-HCT [9]. Future improvements in RIC allo-HCT should aim to further reduce the TRM with an ultimate goal of improving OS.

Donor lymphocyte infusion

Efficacy of adoptive immunotherapy has also been demonstrated by administering donor lymphocyte infusion (DLI) to subjects with MM relapsing after an allo-HCT. A systematic review by El-Jurdi *et al.* showed a pooled overall response rate (ORR) and CR rates of 51 (95% CI: 43–59%) and 26% (95% CI: 19%–33%), respectively, when using DLI for treatment of relapsed MM after allo-HCT [6]. The reported incidence of acute GVHD developing after DLI administration was 53% (95% CI: 44–63%), representing a major source of morbidity and mortality from the procedure [6]. This analysis, however, has several limitations including the lack of uniform criteria among the pooled studies and the absence of data pertaining to disease-specific prognostic factors such as adverse genetic or molecular abnormalities, among other reasons [6]. Moreover, the optimal dose of DLI remains to be defined.

Tumor-associated antigens

Tumor associated antigens (TAAs) are antigens that are tumor-specific, display a tumor-restricted pattern or are overexpressed to a great degree relative to normal tissue, making them ideal targets for peptide/adjuvant vaccines or cellular immunotherapy. An ideal TAA for immunotherapy is functionally important to prevent alternative clonal progression, has limited expression in normal tissues to minimize toxicity when targeting, is expressed in large fractions of myeloma patients to improve applicability, is not downregulated and not shed into the circulation. Functionally competent T cells that are specific to TAAs have been detected in the blood of MM patients, highlighting both their promising role as targets and the conundrum of MM immune evasion [10,11]. The pace in discovery of immunogenic targets for MM immunotherapy is rapidly accelerating. Commonly discussed target antigens include idiotype (Id) myeloma immunoglobulins, MUC1, WT1, cancer testis antigens (such as MAGE and NY-ESO-1), RHAMM, DKK1, HM1.24 and survivin. Almost all MM cells express Id, RHAMM, DKK1 and HM1.24 [12–15]. A majority of MM samples express MUC1, WT1 and MAGE-C1 [16–19]. Cancer testis antigens and survivin are expressed at varying frequencies; however, they are promising for immunotherapy targeting. Here, we provide a brief list of a few TAAs being pursued as therapeutic targets for MM. Details regarding the clinical applications targeting these TAAs with immunotherapy are summarized in later sections, including blockade of immune system checkpoints and vaccine therapies.

Idiotype

The unique immunological properties of any individual immunoglobulin derived from genetic events, including the antigen receptor gene rearrangement process and somatic hypermutation, are referred to as the Id. The enormous immunoglobulin diversity provides unique epitopes that may elicit a specific immune response. Only one specific immunoglobulin is synthesized by a normal B-cell clone and the tumor-specific Id was proposed as a TAA in MM over 40 years ago [20]. Induction of antimyeloma immunity

through immunization with a myeloma Id has been extensively studied in the MOPC-315 murine plasmacytoma model [21]. Vaccinations with tumor-derived paraprotein protected syngeneic mice against a subsequent challenge with MOPC-315 cells [21]. In MM patients, Id-specific $CD4^+$ and $CD8^+$ T-cell responses are demonstrable after *in vitro* stimulation with autologous paraprotein [22]. Id-specific cytotoxic T-cell activity against autologous myeloma cells has been shown after stimulation with Id-loaded dendritic cells (DCs) [23,24]. Selected clinical studies evaluating Id-presenting DC vaccinations in MM are summarized in Table 1.

DKK1

DKK1 is a protein that is secreted and impedes bone formation by the inhibition of the Wnt/β-catenin pathway, thus contributing to the pathogenesis of osteolytic myeloma bone disease [25]. HLA-A2-restricted peptides from DKK1 have been identified and specific cytotoxic T cells against DKK1 have been identified in MM patients, although at a low frequency [15]. Autologous DCs loaded with DKK1 peptides could potentially generate specific T cells, which are able to lyse DKK1-expressing myeloma cells in an HLA-A2-restricted fashion [15].

MUC1

Physiologically, MUC1 is a highly glycosylated epithelial mucin, which is ubiquitously expressed on the luminal surface of most epithelial cells. However, it is overexpressed and aberrantly glycosylated (or underglycosylated) in malignant cells [26]. MUC1 may be recognized by cytotoxic T cells in a MHC-unrestricted fashion [27]. Functionally competent and MUC1 peptide-specific, $CD8^+$ T cells have been detected in patients with MM.

RHAMM

RHAMM, or CD168, is involved in the formation of the mitotic spindle and signal transduction, and is normally expressed in the testis, placenta and thymus [28]. RHAMM is expressed in 100% of MM cases [13], and $CD8^+$ T cell responses are demonstrable [29,30].

WT1

WT1 is a zinc finger transcription factor overexpressed in myeloid malignancies. WT1 is also expressed in lymphoid malignancies and lysis of myeloma cells via WT1-specific cytotoxic T cells has been demonstrated [31].

HM1.24

HM1.24, also known as CD317, BST2 or tetherin, is a cell surface molecule involved in cell signaling, viral infection control and is overexpressed in MM cells. HM1.24-specific cytotoxic T cells have been shown in MM patients [32,33]. HM1.24 was originally thought to be preferentially expressed on terminally differentiated B cells and overexpressed in MM cells; however, a study using tissue microarray found expression of HM1.24 in various normal tissue types questioning the prior notion of skewed expression pattern [34].

Cancer testis antigens

Increased expression of cancer testis antigens, which are normally only expressed in the testis and placenta trophoblasts in physiologic conditions, can also be seen in MM cells. Cancer testis gene expression may increase further with advanced MM and in the presence of cytogenetic abnormalities [10,17,35]; and T cells specific to cancer testis antigens have been detected in the peripheral blood of myeloma patients [10,11].

NY-ESO-1

Spontaneous humoral immune and cellular responses (i.e., T cells) directed against NY-ESO-1 have been detected in MM patients. Specific cytotoxic T cells, expanded by autologous APCs pulsed with NY-ESO-1-derived peptide analog, are able to lyse primary MM cells [10].

MAGE-C1

MAGE-C1 is a frequently expressed cancer-testis antigen in 70–80% of MM. $CD8^+$ T cells against MAGE-C1 have been detected and T-cell responses were specific to those patients expressing *MAGE-C1* mRNA in MM cells [36]. HLA-A2-restricted epitopes have been found from MAGE-C1, and these $CD8^+$ T cells were capable of recognizing MM cells expressing MAGE-C1 [37]. Additionally, specific anti-MAGE-C1 antibodies have been detected in half of MM patients and in almost all patients expressing MAGE-C1 [38].

Ropporin

Ropporin is a recently discovered cancer testis antigen, which appears to be located on the surface of MM cells and ropporin-specific antibodies have been detected in the serum of MM patients [39]. Ropporin was expressed in 44% of MM patients. When incubated with autologous DCs loaded with ropporin, specific cytotoxic T cells showed cytolytic activity against autologous MM cells [40].

Survivin

Survivin is an inhibitor of an apoptosis protein that is overexpressed in MM cell lines and primary MM cells, and is minimally expressed in normal adult tissues [41]. The survivin protein is not detected in healthy individuals, but it is expressed in 41% of newly diagnosed MM patients and 58% of relapsed/refractory patients, suggesting that it might play an important role in MM resistance to chemotherapy [42]. Cytotoxic T cells specific for survivin have been demonstrated in MM patients [43]. Immunization of mice with a DC-based full-length survivin vaccine induced an effective immune response against malignancy, without appreciable hematopoietic toxicity [44].

The growing interest in cancer stem cell hypothesis may provide explanation for incurability in the majority of myeloma patients [45]. Although precise phenotypes of MM cancer stem cells remain unclear, studies have examined the B-cell nature of tumorigenic MM cells and demonstrated that these cells lack expression of CD138 [46,47]. TAAs on these cells have not been characterized thoroughly, although they are likely to express at least some of the

identified MM TAAs, such as survivin [48]. Further studies are needed to evaluate whether immunotherapy targeting prevalent myeloma TAAs could eradicate MM 'cancer stem cells'.

Therapeutic monoclonal antibodies against MM

The past two decades have brought significant advances related to development of monoclonal antibodies (mAbs) for the treatment of various neoplasms [49–51]. Use of mAbs in combination with conventional chemotherapy has become the standard of care for various diseases [52]. For a mAb-based therapy to be effective against MM, the target antigen should be expressed in a high percentage of neoplastic plasma cells, components of the bone marrow microenvironment such as marrow stromal cells, or cells of the immune system that mediate immune response [53,54]. The chimeric mAb against CD20, namely rituximab, is a well-established therapy for B-cell lymphomas and has been investigated for MM. Several alternative mAbs are in preclinical and clinical studies. Here, we summarize results of clinical trials with several promising mAbs, namely daratumumab, siltuximab and elotuzumab. Other mAbs are also undergoing preclinical and clinical evaluation in MM. Discussion of blocking antibodies against immune system checkpoint regulators such as those targeting CTLA-4 (ipilimumab), PD-1, and PD-L1 will be discussed separately in the section 'Interrupting negative regulation of the immune system', below.

Rituximab (anti-CD20)

Studies evaluating the efficacy of rituximab in MM have resulted in disappointing outcomes. CD20 may be expressed in only 30% of plasma cells in myeloma patients and expression has been associated with a small mature plasma cell morphology and t(11;14) [55,56]. A Phase II study by Treon *et al.* included 19 patients with previously treated MM who received weekly rituximab 375 mg/m^2 for four doses and demonstrated an objective response of only 5% (partial response [PR]: 5%) and evidence of stable disease [SD]: in five (26%) subjects [57]. Moreover, a multicenter Phase II study in France, using the same schedule of rituximab [57], described a minor response in only one (7%) and SD in five (36%) of 14 subjects treated [58]. Interestingly, 100% of plasma cells expressed CD20 in the subject achieving a minor response, suggesting that the level of CD20 expression might, to some extent, play a role in predicting responses [58]. Baz *et al.* reported that addition of rituximab to melphalan prednisone did not improve ORR or survival in MM [59].

It is possible that other factors such as dissociated action of complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity, polymorphisms in FCγRIIIA receptor and perhaps inadequate dosing schedule might explain the poor responses observed with rituximab [57,58].

Daratumumab (anti-CD38)

CD38 is a type II transmembrane glycoprotein that is highly expressed in various hematologic malignancies including MM, where CD38 is expressed in almost all patients [60]. de Weers *et al.* demonstrated that daratumumab, a novel high-affinity therapeutic human mAb against a unique CD38 epitope, was capable of inducing potent antibody-dependent cellular cytotoxicity in CD38-expressing lymphomas, as well as myeloma-

derived cell lines using both autologous or allogeneic effector cells [61]. Additional preclinical investigations have shown that cell-mediated cytotoxicity of primary myeloma cells was augmented when daratumumab was combined with lenalidomide [61]. A dose escalation Phase I/II study evaluated five dose levels (≤1, 2, 4, 8, 16 and 24 mg/kg) of daratumumab in 32 subjects, median age 61 (42–76) years, with relapsed/refractory MM [62]. Infusion-related reactions were seen in 9% of patients during the predose infusion and in 26% during the first infusion, with a decrease in frequency with subsequent infusions. Investigators reported five late reactions (bronchospasm in two, headache in one, dyspnea in one and fever in one). The authors did not observe changes in hemoglobin or platelet counts over time [62]. A reduction in paraprotein was observed in 15 (47%) of 32 subjects: PR (n = 4), minimal response (n = 6) and SD (n = 5) [62]. A Phase I/II open-label international multicenter trial is currently investigating the safety of combining daratumumab with lenalidomide plus dexamethasone in patients with relapsed/refractory MM [201].

Siltuximab (anti-IL-6)

IL-6 is a cytokine that plays a central role in the growth and survival of MM cells within the bone marrow milieu making it an attractive therapeutic target [63]. Siltuximab is a chimeric anti-IL-6 mAb, which has been evaluated alone and in combination with dexamethasone in patients with relapsed/refractory MM [64]. Of the 49 subjects who received combination therapy, all experienced at least one adverse event, with the most common nonhematologic toxicities being fatigue (43%) and abnormalities in hepatic function (31%) [64]. Hematologic toxicities were common: thrombocytopenia (49%), anemia (35%) and neutropenia (29%). Infection-related adverse events were commonly reported, occurring in 57% with siltuximab plus dexamethasone-treated patients, including ≥grade 3 infections in 18%. The combination of siltuximab plus dexamethasone in 47 evaluable subjects resulted in a median time-to-response of 1.6 (range: 0.7–8.1) months and an ORR of 19% (PR: 19%) using the International Myeloma Working Group criteria [64]. A randomized, double-blind, placebo-controlled Phase II study comparing siltuximab plus bortezomib versus bortezomib plus placebo in 286 patients with relapsed/refractory MM did not improve PFS or OS with addition of siltuximab [65]. However, a higher frequency of serious adverse events was reported in the siltuximab arm (29 vs 24%) [65]. This therapy appears promising, however, further studies are needed with attention to better address infection-related toxicities.

Elotuzumab (anti-CS1)

CS1 is a member of the signaling lymphocyte-activating molecule family of cell surface receptors, which is commonly expressed at high levels on myeloma cells [66]. Elotuzumab is a humanized IgG1 mAb, which targets CS1. Moreau *et al.* presented results of the combination of elotuzumab, lenalidomide plus dexamethasone used in 73 patients with relapsed/refractory MM [67]. A total of 50% of enrolled subjects received a dose of 10 mg/kg of elotuzumab, while the remaining received 20 mg/kg. Overall, 92% of subjects treated with the lower elotuzumab dose achieved an objective response compared with 76% treated with the higher dose. A PR or better was observed in 91% of subjects who had received no more than one previous line of therapy [67]. Two randomized Phase III trials evaluating lenalidomide plus dexamethasone with or without eloutuzumab in previously

untreated (ELOQUENT-1) or in relapsed/refractory MM (ELOQUENT-2) are currently ongoing [202,203].

Encouraging results associated with new-generation mAbs are likely to result in their incorporation in the future treatment regimens for MM. Other mAbs apart from the ones mentioned herein are at various stages of development.

Clinical trials of vaccines utilizing myeloma-associated antigens

Several TAAs have been examined in clinical trials as therapeutic targets for plasma cell disorders. In many cases, promising immune reactivity seen with peptide/protein vaccines against TAAs are being expanded by using cell-based approaches and immune system modulation.

Id vaccines

Clinical trials evaluating the Id as a vaccine have been conducted by numerous investigators (Table 1) and have shown that such vaccines can induce both cellular and humoral immunity [68], and that they may induce a reduction in circulating tumor cells [69]. Moreover, they may also induce immune responses despite immunosuppressive effects of prior high-dose chemotherapy [70] and that loss of oligoclonality correlates with remission duration [70]. These trials were conducted under various conditions including the inclusion of GM-CSF, IL-12, keyhole limpet hemocyanin and at various stages of disease. No clinical benefit has been proven with this approach thus far. Additional studies using the Id together with DCs as vaccines have been conducted and will be discussed below.

TAA peptide vaccines

Some trials using peptide vaccines in myeloma patients have been reported, while others are underway. The most robust HLA-A2-restricted epitopes within RHAMM, designated at R3, was targeted in peptide vaccine trials for MM and other hematologic malignancies. T-effector (T-eff) activity was demonstrated, as well as a reduction in free light chains in several patients [30].

Interesting results of a peptide vaccine strategy testing different HLA-A2-restricted MUC1 peptides injected along with CpG and Montanide™ adjuvants led to a decrease in tetramer-positive vaccine-specific T cells for MM patients. No clinical responses were observed and several patients were noted to have increased Tregs prompting investigators to conclude that use of these adjuvants might promote negative regulatory factors [71]. An alternative MUC1 peptide, encoding the complete signal peptide domain, is able to bind multiple MHC class I and class II alleles, and induce both CD4 and CD8 responses *in vitro* [72]. Ongoing clinical trials in MM patients await peer review, yet this strategy holds promise to expand therapy eligibility for those without HLA-A2.

A case report of a myeloma patient receiving WT-1 vaccine demonstrated decreased bone marrow plasma cells and urine monoclonal protein accompanied by a $CD8^+$ tetramer response following 12-weekly intradermal injections of an HLA-A*2402-restricted WT1 peptide combined with montanide adjuvant as monotherapy [73]. Emergence of WT1-

specific cytotoxic T cells was noted after DLI following T-cell-depleted allogeneic HCT in MM patients, supporting the use of WT1-specific immunotherapy approaches [74]; however, controlled trials specific for myeloma patients are otherwise lacking for this TAA.

Proposed and ongoing clinical trials for plasma cell dyscrasias include naked or adjuvant-coupled peptide vaccines. Examples include PVX-410, consisting of four peptides [75,204], MAGE-A3 and NY-ESO-1 vaccine [205], and a WT1 vaccine [206].

Cellular therapy

DC vaccines

Many groups have explored DC-based therapeutic vaccine strategies against MM. This includes exploration of DC-aided targeting of the Id or whole-tumor-derived TAA. In clinical trials, immune responses against tumors are repeatedly demonstrated, however, newer strategies are likely required to potentiate these immune responses into a demonstrable clinical benefit. Specific TAA peptide or protein-loaded DC vaccines remain in the preclinical realm.

Id-DC vaccines have been evaluated extensively. These vaccines are able to induce Id-specific cytotoxic T lymphocyte (CTL) and Thl T-cell responses [76], induce anti-Id antibody responses [77] and produce immunologic response in 45% of vaccinated patients (five of 11) when given with GM-CSF [78]. In a Phase I/II clinical trial, effective T-cell responses were elicited by subcutaneous injections of cryopreserved DCs loaded with either patient-specific tumor Id whole protein or Id (VDJ)-derived class I-restricted peptides [79].

One strategy to promote the Id vaccine response is to use CD40L-matured DCs. In nine patients with smoldering or stable MM, autologous DCs were pulsed with Id and keyhole limpet hemocyanin then matured with cytokines and CD40L, and injected intranodally. Both cellular and humoral immune responses against Id were elicited, and 56% of patients mounted an Id-specific CTL response [80]. This suggests that Id-pulsed DC vaccines can elicit tumor-specific immune responses, however, these trials did not prove a definitive clinical benefit [4].

Alternatively, whole tumor cells can also be used to generate TAA vaccines. Such a strategy allows recognition of multiple epitopes and an opportunity for polyclonal T-cell responses. DCs loaded with lysed or irradiated purified MM cells can expand IFN-γ-producing T cells cytotoxic against primary MM cells [81–83]. Preclinical data indicate that this strategy may be preferable over DC-Id vaccine in the induction of antitumor immunity [84]. Rosenblatt *et al.* conducted a Phase I study which demonstrated that an autologous DC/myeloma fusion vaccine could safely be administered to MM patients resulting in induction of tumor-specific T cells [85]. Patients were required to have at least 20% plasma cells in the bone marrow. Three injections at 3-week intervals, up to 4×10^6 fusion cells per dose, were administered. While injection site reactions were common, serious adverse events were limited. Eleven of 16 evaluable patients exhibited SD after vaccination and optimization for this strategy is ongoing.

Preclinical data point to promising strategies utilizing myeloma-derived RNA [86] or HSP gp96 loaded onto DCs [87]. The latter may bypass the need for autologous tumor via use of allogeneic cell line-derived HSP gp96. Non-DC, APCs, may also gain favor against MM, such as *ex vivo*-activated B cells expressing CD40 loaded with MM lysate [88].

Future DC vaccine strategies for myeloma are likely to benefit from such novel approaches. Strategies under clinical development to improve DC vaccines include combination with autologous transplant, cytokine administration or drugs that inhibit negative regulation, and these approaches will be discussed separately.

NK cells

NK cells can help activate DCs and provide immune surveillance against tumor growth. Current translational activities of NK cell therapy for myeloma involve the use of allogeneic NK cells to mediate immune responses. Haplo-identical NK cells infused after fludarabine plus melphalan conditioning led to transient donor chimerism prior to engraftment of autologous hematopoietic cells. An antidonor response was likely responsible for the loss of the haploidentical NK cells, limiting further application of this approach [89]. Future focus areas of NK cell therapies for myeloma include the procurement of NK cells from cord blood units, cytokine/ligand enhanced expansion of autologous NK cells [90], or myeloma-activated NK cells [91].

T cells

An alternative cell therapy approach entails the adoptive transfer of T cells into patients. These strategies variably involve *ex vivo* expansion of marrow-infiltrating lymphocytes (MILs), T cells primed for TAA peptides, T cells transduced to express a TAA-specific transgenic T cell receptor (TCR), or T cells induced to express a TAA epitope specific chimeric antigen receptor (CAR). The diversity of the preclinical approaches is remarkable, however, to date, only a few of these approaches have been attempted and reported as therapy for MM.

Preclinical evidence suggests MILs have a greater capacity to recognize myeloma TAA compared with peripheral blood T cells [92]. Current efforts to expand this strategy into the clinical setting are ongoing [207]. In the first report of MIL therapy for MM, autologous MILs were expanded with anti-CD3/CD28 beads for 7 days and infused on day +3 after stem cell infusion following standard high-dose melphalan conditioning. Patients achieving a CR/PR showed greater bone marrow $CD8^+$ numbers at day +60 compared with patients with SD or PD. In patients with SD or PD, the immune infiltrate in the bone marrow was characterized by large numbers of effector and effector memory T cells and few $CD8^+$ central memory cells at baseline. This was an interim report of the first 22 patients and safety data revealed a self-limited skin rash, attributed to autologous GVHD, was seen in 32% and lymphoid recovery was adequate with a mean absolute lymphocyte count on day +15 of 886 cells/μl [93].

Interim results were recently reported of a strategy where patient T cells were genetically engineered to express an affinity-enhanced TCR (HLA-A0201-restricted) targeting an epitope shared by the NY-ESO-1 and LAGE-1 tumor antigens [94]. Sixteen patients with

high-risk or relapsed myeloma were reinfused with their own *ex vivo* transduced and expanded T cells 2 days after stem cell infusion following high-dose melphalan. By day +100, 27% of patients had experienced a grade 3–4 serious adverse event possibly or probably related to treatment. Several patients had biopsy-proven immune enteritis, possibly mediated by infiltrating engineered cells, and all appeared to improve with steroid therapy. Trafficking to and persistence of marrow cells was observed, and the engineered cells were detected in all patients at their latest timepoints tested by quantitative PCR. Interestingly patients that progressed had weak or no expression of LAGE-l/NY-ESO-1, suggesting specific targeting of tumor cells [94].

Because adoptive transfer of T cells is enhanced by a lymphodepleted state, many of these efforts are built upon a platform of standard high-dose chemotherapy and auto-HCT, and results of respective clinical studies will be discussed in the ‘High-dose chemotherapy and auto-HCT as a platform for immunotherapy’ section.

CART cells

T cells may be genetically engineered *ex vivo* to express a CAR in order to direct autologous T cells against a desired target. CD19 CAR T cells, engineered to express a CAR against CD19, were recently shown to provide clinical benefit to chronic lymphocytic leukemia and B-cell acute lymphoblastic leukemia patients in small feasibility studies [95].

A CAR consists of a TAA-binding domain, an extracellular spacer region, a transmembrane domain and an intracellular signaling domain. Most CAR-binding domains are scFv derived from a mAb against the desired TAA. The intracellular signaling domain typically mediates T-cell activation through the tyrosine-based-activating motif of the CD3ζ chain. First-generation CAR signaling domains consist only of the TCR signaling machinery, such as CD3ζ therefore, upon binding, they are stimulated as if the T cell had bound its cognate target absent costimulatory signals mediated through CD28, CD137 (41BB) or CD134 (OX40). These signals are typically provided by APCs to facilitate avoidance of T-cell anergy, which can render T cells immunologically ineffective. Second-generation CARs were introduced to overcome this limitation by including one of the costimulatory signaling domains, such as CD28 or 41BB, in addition to the CD3ζ chain. Third-generation CARs include two of the costimulatory intracellular signaling domains in addition to the TCR signal. The CAR is typically introduced *ex vivo*, by a lentivirus or adenovirus construct, into T cells collected from the patient via apheresis. Prior to infusion into the patient, those cells are expanded by anti-CD3 plus anti-CD28 or IL-2. Optimal proliferation and persistence of the CAR T cells likely require some degree of preinfusion chemotherapeutic lymphodepletion, such as pentostatin and cyclophosphamide.

This therapy is not without risks. With genetic engineered therapy, the introduced gene carries risks of uncontrolled cell expansion and has potential for tumor formation, leading some groups to insert suicide genes. As the CAR is inserted by a viral vector there is the risk that the virus might replicate *in vivo*. In addition, as with any targeted therapy, expression of the TAA on nontumor cells invites unwanted toxicity. With CAR T-cell therapy, this might be magnified by the release of proinflammatory cytokines and further damage to normal tissues.

This technology remains experimental and the full clinical applicability and limitations remain unknown. Importantly, little work has been carried out to expand CAR therapy to the realm of MM. Preclinical work suggests that B-cell maturation antigen (BCMA or CD269) could be a suitable target for CAR therapy for MM [96].

Interrupting negative regulation of the immune system

Numerous pathways, receptors and signaling molecules are implicated in turning off the immune system to avoid unperturbed autoimmunity. Blocking these mechanisms to promote antitumor immune responses is an increasingly attractive strategy. Several of these are at the clinical or late preclinical stages and will be reviewed here.

Cell surface signal blockade (CTLA-4 or PD-1)

CTLA-4 is a critical negative regulator of immune activation. T-cell response is determined by the balance between positive signaling through CD28 and negative signaling through CTLA-4 [97]. The CTLA-4-blocking antibody ipilimumab is FDA approved as a therapy against metastatic malignant melanoma. Myeloma patients have increased expression of CTLA-4 on $CD4^+$ and $CD8^+$ T cells and MM tumor cells exhibit increased expression of the CTLA-4 ligand, CD86 [98]. Preclinical data suggest that CTLA-4 blockade exerts a significant immune-mediated antimyeloma effect [99]. This agent is being considered as a therapeutic option against myeloma.

PD-1 is another cell surface receptor that negatively regulates T-cell responses, via interaction with its ligands PD-L1 and PD-L2, which may be expressed by tumor cells. Several different PD-1- and PD-L1-blocking antibodies are at varying stages of development as cancer therapeutics. NK cells also express PD-1 and blockade has shown to enhance human NK cell activity against autologous myeloma tumor *in vitro*, and combination with NK cellular therapy could prove of benefit [100]. Interim results of a clinical trial of CT-011 (anti-PD-1 mAb) in combination with autologous transplant were recently reported. CT-011 appears safe for further exploration and may promote cellular antitumor immune activity against MM [101].

Alteration of the T-cell repertoire (Treg/Th17)

Different T-cell subsets perform alternating roles in suppressing or activating the immune response. Tregs are a $CD4^+/CD25^+/FOXP3^+$ subset of T cells that play a key role in maintaining self-tolerance. Both *in vitro* and *in vivo*, Tregs suppress immune responses. Tregs are present in increased numbers in solid and hematologic malignancies where they suppress tumor-specific T-eff responses [102]. Treg biology is complex and incompletely understood, although CTLA-4 and PD-1 signaling may play a role, highlighting the interplay between these regulatory mechanisms. MM patients harbor tumor-specific Tregs, which blunt T-effs against myeloma [103], and as patients with MM undergo disease progression, the number of peripheral blood Tregs correspondingly increases due to peripheral expansion while maintaining a high degree of suppressive capacity [104]. Alternative data report dysfunction of Tregs in MM patients, however, the lack of exclusion of T-effs from the samples has been implicated as a reason for this contradictory finding

[105]. Borrello and colleagues demonstrated a reduction in the ratio of Tregs to conventional T cells in MM patient bone marrow as compared with healthy control bone marrow. Interestingly, this was accompanied by a corresponding increase in circulating Tregs for MM patients [106]. This is in contrast to studies of mRNA expression of FoxP3 in the bone marrow of MM patients, suggesting that suppressive Treg numbers are increased in the marrow environment compared with healthy controls [107]. The intricacies of Treg suppression of antitumor immune response against MM and the importance of bone marrow Tregs versus circulating Tregs remains unclear, although strategies to decrease Treg numbers in the post-transplant setting are underway and will be discussed in the 'High-dose chemotherapy and auto-HCT as a platform for immunotherapy' section.

Alternately, it was demonstrated that the Th17 subset of T cells is increased in MM patient's bone marrow and likely contributes to osteolytic lesions. Th17 cells are a T-helper cell subset characterized by the RORγt transcription factor. They produce IL-17, are considered highly inflammatory, act to recruit neutrophils, and may promote antitumor T-cell activity. TGF-β and IL-6 are cytokines expressed to a high degree in MM bone marrow and they both promote Th17 differentiation. It has been proposed that blockade of IL-17 signaling or STAT3, the transcriptional regulator of RORγt, might reduce MM osteolytic bone disease.

Studies in preclinical models have shown that Treg removal from the tumor microenvironment promotes tumor eradication via amplification of a T-eff response [108,109].

Modulation of myeloid-derived suppressor cells

An immature myeloid cell population works to suppress immune responses in cancer patients. These myeloid-derived suppressor cells (MDSCs) are heterogeneous in nature. Recent work by Gorgun *et al.* revealed the MDSCs were increasingly present in the peripheral blood and the bone marrow of patients with MM compared with healthy donors [110]. MDSCs suppress T-cell activity and promote myeloma tumor growth, while MM cells promoted MDSC generation from donor peripheral blood [110]. Interestingly, MDSCs were shown to have a capacity to differentiate into functional osteoclasts, suggesting a link to a premetastatic niche for MM cells within the bone [111]. The increased tumor burden mediated by these cells is mitigated by bisphosphonate therapy [111]. Other groups have shown alternative mechanisms for immune potentiation with bisphosphonates and extended clinical investigations into the immune potentiation ability of this well-accepted therapy are warranted [112]. Preclinical work to skew or alter MDSCs away from suppressive activity is ongoing.

Interfering with bone marrow microenvironment & stromal interactions

The close interplay between malignant plasma cells and protective bone marrow microenvironment niche has been known to provide a tumor survival advantage and emergence of drug resistance [113,114]. MM cell adhesion to bone marrow stromal cells mediated through multiple adhesion molecules and pathways, including, but not limited to, VLA-4, ICAM, IL-6, DKK1, IGF-1, CXCL12–CXCR4 axis and CS-1, contributes to MM cell proliferation [113]. There are several potential therapeutic targets for the disruption of

plasma cell–bone marrow stromal interaction. For example, *in vivo* models have shown antiangiogenic properties of immunomodulatory agents such as thalidomide [115], suppression of the stromal production of IL-6 by histone deacetylase inhibitors [116] and blockade of DKK1 signaling cascade via a mAb [117]. Perturbation of MM cell microenvironmental interactions can be also achieved by a small molecule inhibitor or antibody against CXCR4 or CS-1. It is expected that the rationale design of future immunotherapeutic approach targeting the complex network of MM–bone marrow stromal interactions may overcome the conundrum of MM drug resistance.

High-dose chemotherapy & auto-HCT as a platform for immunotherapy

By depleting lymphocytes, high-dose melphalan creates ‘space’, which promotes rapid proliferation of T cells transferred within the auto-HCT graft. Because of this effect, termed homeostatic repopulation, auto-HCT holds significant promise as a platform for immunologic therapies [118]. Homeostatic repopulation is known to amplify tumor-specific T-cell responses in cancer patients [108,118–120]. Furthermore, this homeostatic repopulation reverses T-cell hyporesponsiveness, or anergy, to TAAs further promoting tumor rejection [119]. Id-DC-based vaccines have been explored in combination with auto-HCT. Adequate T-cell recovery and antimyeloma T-cell activity in the setting of homeostatic repopulation after transplantation have been demonstrated [121,122]. Similarly, adoptive T-cell strategies allow for expanded T-effs to be transferred back to the patient after high-dose chemotherapy and the unfractionated or CD34 selected cell product.

For MM patients, *ex vivo* costimulated T-effs infused into patients after high-dose melphalan and auto-HCT were able to induce antibody responses compared with controls that did not receive these expanded T-effs [123]. This work has now been expanded to the evaluation of several TAA peptide vaccines. Rapoport *et al.* reported that an HLA-A2-restricted combined peptide vaccination, consisting of the survivin Sur1M2 peptide (LMLGEFLKL) and three hTERT peptides, could elicit an immune response in the context of auto-HCT for MM [124]. In this trial, 28 MM patients were vaccinated prior to collection of T cells by apheresis. These cells were then stimulated and expanded *ex vivo* prior to reintroduction into the patient during the lymphopenic period after high-dose chemotherapy and auto-HCT. Patients were then revaccinated after transplant to potentiate that immune response. Although patients were not stratified by TAA expression, it was demonstrated that adoptive transfer of vaccine-primed and costimulated T cells increased cellular antitumor immune reconstitution in the post-transplant setting. Reactions to the peptide vaccines were acceptable with side effects limited to muscle aches, redness and induration at the injection site [124]. A second peptide vaccine utilized in this fashion is GM-CSF and poly-ICLC mixed with a multipeptide MAGE-A3 vaccine containing two HLA-A2-restricted class I peptides, and a promiscuous class II peptide linked to HIV-1-TAT sequence to enhance peptide presentation [125]. Patients were vaccinated, then these T cells were collected by leukapheresis and costimulated and expanded *ex vivo* using anti-CD3/anti-CD28 mAb-conjugated beads. Interim results were recently reported and revealed that in the 25 patients treated, vaccinations were associated with injection site reactions in the majority of patients. The protocol was modified to exclude montanide as an adjuvant following the development of sterile abscesses in two patients. MAGE-A3 induced cytokine production *in vitro* in 79% (11

out of 14) patients with responses peaking at day 100–180. Interestingly, MAGE-A3 antibody responses were pronounced in seven of nine patients receiving montanide adjuvant, but in zero of the seven that did not [125].

Little attention has been paid to the inherent, nonexpanded, T-effs present within the unselected cytokine mobilized stem cell graft and it has been established that following auto-HCT, competent T-effs persist [126]. Simple immune manipulation, without complex *ex vivo* expansion or vaccination, might lead to increased responses after standard auto-HCT, and such strategies are being explored [208].

Another promising strategy with auto-HCT entails depletion of Tregs. Studies in preclinical models have shown that Treg removal from the tumor microenvironment promotes tumor eradication via amplification of a T-eff response and that combining Treg depletion with homeostatic repopulation can further promote that response [108,109]. Depleting Tregs from the autologous stem cell graft may promote untamed repopulation of MM-specific T-effs and enhance the immune response against myeloma. Post-transplant administration of anti-CD25 mAbs may promote an antitumor effect after transplant, as evidence by allogeneic transplantation studies suggesting a decreased relapse rate and increased incidence of GVHD [127]. Alternatively, mechanical methods of depleting CD25 cells from the stem cell inoculum decreases Tregs and maintains adequate CD4 and CD8 central memory and naive cells, important subsets for prolonged antitumor immunity [Locke FL, Unpublished Data]. Similar strategies to deplete Tregs before the *ex vivo* expansion of T cells are currently employed [94].

Evidence shows that simple immune modulation via post-auto-HCT maintenance therapy with immunomodulatory agents or a proteasome inhibitor prolongs remission duration and possibly improves survival [128–130], however, more aggressive immune manipulation may be needed for patients with active disease at auto-HCT (e.g., those with less than very good partial response). Current trials now must make allowance for the use of lenalidomide maintenance after auto-HCT, considering both the clinical benefit and the possible immune potentiation of an antitumor effect.

Disruption of negative regulation via blocking antibodies against PD-1, PD-L1 or CTLA-4 in combination with lymphodepletion and auto-HCT has been evaluated in the preclinical realm, revealing that such an approach can be synergistic. Bluestone and colleagues demonstrated that homeostatic proliferation combined with CTLA-4 blockade can promote antitumor immunity in a murine model of myeloma [131]. Objective responses were demonstrated when ipilimumab was administered to patients with relapsed hematologic malignancies after allogeneic transplant [132], and clinical trials are ongoing to evaluate the effectiveness of such a combination after donor transplant for diseases including myeloma [209]. Such an approach has not been reported for autologous transplant patients with ipilimumab. Early results of a clinical trial of CT-011, an anti-PD-1 mAb, at 3 mg/kg intravenous once every 6 weeks × three doses initially administered 1 week after high dose melphalan conditioning auto-HCT, suggests that this combination strategy is tolerable, with diarrhea being a common toxicity. Furthermore, this strategy appears to expand myeloma TAA specific T cells in both the peripheral blood and bone marrow. Anticipation surrounds

the results of a second cohort of patients that, in addition to CT-011 and transplant, will also receive the previously mentioned autologous DC/myeloma fusion vaccine [101].

Conclusion & future perspective

Recent success with immunotherapies for various neoplasms, combined with a plethora of novel strategies against myeloma, heightened excitement at the prospect of improving clinical outcomes for myeloma by improving antitumor immunity. Increased understanding of myeloma TAA, availability of more potent vaccines, expanded immune-modulating therapies, development of agents that block immune-suppressive pathways, increased sophistication of adoptive cell therapy techniques and capitalization upon standard autologous transplant are all important standalone or combination strategies that could ultimately improve prognosis in patients with MM. Moreover, therapeutic mAbs being evaluated at the present time in MM appear to be showing encouraging efficacy and it is likely that they will become an integral component of the therapeutic armamentarium to be used for treatment of MM in the future. Several questions remain unanswered, including the ability of immune-based therapies to alter the natural history of the disease, particularly when adverse molecular and genomic prognostic factors are present.

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Executive summary

Allogeneic hematopoietic cell transplantation demonstrates evidence of immune control in multiple myeloma

- Evidence suggests that allogeneic hematopoietic stem cell transplantation and donor lymphocyte infusions exert antimyeloma immune effects.
- This therapy prolongs disease-free survival for patients with multiple myeloma, hence supporting pursuit of future T-cell immunotherapeutic strategies.

Myeloma-associated tumor-associated antigens

- Functionally competent T cells specific to myeloma tumor-associated antigens have been detected in the peripheral blood of patients with myeloma. Myeloma-specific tumor-associated antigens have been characterized extensively and are of interest as targets for immunotherapeutic strategies.
- Tumor associated antigens of special interest for myeloma immunotherapy are idiotype, MUC1, cancer testis antigens, RHAMM, WT-1 and survivin.

Therapeutic monoclonal antibodies against multiple myeloma

- Various monoclonal antibodies have shown promising early results in patients with multiple myeloma, namely daratumumab (anti-CD38), siltuximab (anti-IL-6), and elatuzumab (anti-CS1), among others.
- Other monoclonal antibodies are at various phases of development.

Vaccination with idiotype or peptide

- Although early idiotype vaccine trials showed promise due to evidence of immunologic activity against myeloma, this strategy has not yet demonstrated improvement in clinical outcomes.
- MUC1, WT-1 and survivin peptide vaccines have been evaluated against myeloma. Clinical benefit from these vaccines may require the combination of vaccination with alternative immunotherapeutic strategies.

Dendritic cell vaccines

- Dendritic cell (DC) vaccines against idiotype have demonstrated both cellular and humoral immune responses against Idiotype. An autologous DC–myeloma fusion vaccine could be safely administered to myeloma patients resulting in induction of tumor-specific T cells. Vaccines using DCs loaded with myeloma derived RNA or non-DC APCs may prove beneficial against myeloma.

Adoptive T-cell immunotherapy

- *Ex vivo*-activated T cells have shown promise as a way to promote antimyeloma tumor-associated antigen responses.

- Ongoing efforts to prove clinical benefit of chimeric antigen receptor T cells against other hematologic malignancies are likely to further spark interest in these therapies against myeloma.

Interrupting negative regulation of the immune system

- Numerous cell surface receptors act to help negatively regulate the immune system, including CTLA-4, PD-1 and PD-L1. Early clinical results with an antibody against PD-1 have sparked interest in such an approach as therapy against myeloma, possibly in combination with autologous transplant and/or vaccination.
- Tregs increase in the peripheral blood of myeloma patients as their disease progresses, although Treg numbers may be decreased within the bone marrow. The functional capacity of Tregs and the role they play in pathogenesis of myeloma is not entirely understood.
- Growing evidence suggests a role of myeloid-derived suppressor cells in the pathogenesis of myeloma, and modulation of this cell subset may prove of therapeutic use in the future.

High-dose chemotherapy & autologous hematopoietic cell transplant as a platform for immunotherapy

- Autologous transplant remains a standard-of-care therapy for myeloma patients. Numerous trials for myeloma have utilized high-dose chemotherapy and autologous hematopoietic cell transplant as a platform for immunotherapy. The lymphodepleted state after high-dose chemotherapy provides ideal conditions for homeostatic repopulation as a way to promote T-cell expansion.

Table 1

Selected clinical studies (n ≥5) evaluating idiotype-presenting dendritic cell vaccinations in multiple myeloma.

Study (year)	n	DC type	Timing of vaccination/adjuvant	Immunologic responses	Ref.
Reichardt *et al.* (1999)	12	Immature DC	HDT followed by Id vaccination/KLH	2/12 Id-specific proliferative response; 1/3 Id-specific CTL response	[121]
Lim *et al.* (1999)	6	Monocyte-derived DC	Three following chemotherapy and three untreated/KLH	5/6 Id-specific proliferative response; 3/6 increased Id-specific CTL precursor frequency	[133]
Liso *et al.* (2000)	26	Immature DC	HDT followed by Id vaccination/KLH	4/26 Id-specific proliferative response	[122]
Titzer *et al.* (2000)	11	$CD34^+$ cell-derived DC	Following chemotherapy (two with HDT)/none (used GM-CSF)	4/10 Id-specific T-cell response by ELISPOT	[78]
Yi *et al.* (2002)	5	Monocyte-derived DC	HDT followed by Id vaccination/IL–2	2/5 Id-specific proliferative response; 4/5 Id-specific T-cell response by ELISPOT	[134]
Reichardt *et al.* (2003)	12	Monocyte-derived DC	HDT followed by Id vaccination/KLH and GM-CSF	2/12 Id-specific proliferative response; 1/12 Id-specific CTL response	[76]
Röllig *et al.* (2011)	9	Mature monocyte-derived DC	Stage l MM/KLH	5/9 Id-specific proliferative response; 8/9 Id-specific cytokine release	[135]

CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; ELISPOT: Enzyme-linked immunosorbent spot; HDT: High-dose therapy; Id: Idiotype; KLH: Keyhole limpet hemocyanin; MM: Multiple myeloma.