

Chapter 2

Novel Antigenic Targets for Immunotherapy in Myeloma

Qing Yi

2.1 Introduction

The American Cancer Society estimates that 20,180 patients will have been diagnosed with multiple myeloma (MM) in year 2010 and 10,650 will die of this disease. These statistics indicate that MM is the second most commonly diagnosed hematologic malignancy after non-Hodgkin lymphoma. Moreover, over the past 25 years, the number of new cases has increased by more than twofold, supporting the importance of this disease as a public health concern [1]. Over the last decade, MM has emerged as a paradigm within the hematologic malignancies for the success of translational medicine. With the bench-to-bedside approaches used by the leaders of this field, four novel drugs have been approved for this disease in the past 5 years, including bortezomib, thalidomide, pegylated liposomal doxorubicin, and lenalidomide. These agents initially were used in the relapsed/refractory setting, and are now being adopted as part of front-line therapy [2], where they appear likely to have even greater benefits. Despite these advances, however, MM remains incurable, and the vast majority of patients eventually relapse with disease that is typically more resistant to therapy than in prior lines of treatment. This indicates that there is a greater need than ever to focus on this disease and to develop more effective therapies. Immunotherapy is an appealing option for this purpose [3].

Q. Yi, M.D., Ph.D. (✉)

Department of Lymphoma/Myeloma, Division of Cancer Medicine, Center for Cancer Immunology Research, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 0903, Houston, TX 77030, USA
e-mail: qyi@mdanderson.org

There is ample evidence to indicate that myeloma cells are susceptible to T cell-mediated cytotoxicity. In the post-allograft relapse setting, in which myeloma patients are chemotherapy refractory, long-lasting disease remission has been achieved after infusion of donor lymphocytes, a phenomenon termed graft-versus-myeloma effect [4, 5]. This graft-versus-myeloma effect is closely associated with graft-versus-host disease, and donor-derived alloreactive and tumor-specific T cells are believed to mediate these effects [6]. These observations strongly suggest that chemotherapy and immunotherapy kill myeloma cells by different modes of action that are non-cross-resistant; therefore, they should work synergistically.

2.2 Myeloma-Specific Antigen: Idiotypic Proteins

Idiotypic proteins are derived from monoclonal myeloma cells and are considered tumor-specific antigen. Active immunization against idiotypic determinants on malignant B cells has produced resistance to tumor growth in transplantable murine B-cell lymphoma and plasmacytoma [7–11]. The presence of idiotype-specific T cells in the peripheral blood of patients with MM or with the benign form of the disease, monoclonal gammopathy of undetermined significance (MGUS), has been studied by detecting idiotype-induced T-cell proliferation and cytokine secretion by using the enzyme-linked immunospot (ELISPOT) assay [12].

Idiotypic-specific T cells at a low frequency were detected in 90% of patients with MM or MGUS [13–15]. Consistent with these results, we and others have shown that T cells in myeloma patients responded to peptides corresponding to complementarity-determining region I–III of heavy and light chains of the autologous M-component [16–19]. We found that idiotype-induced T-cell stimulation was mainly confined to the CD4⁺ subset in most of the patients examined and was MHC class II-restricted. Idiotype-specific CD8⁺ T cells were also demonstrated, but at a lower frequency. Idiotype-specific CD4⁺ and CD8⁺ T cells were mainly of the type-1 subsets, as judged by their secretion of interferon (IFN)- γ and interleukin (IL)-2 [20, 21]. Moreover, the proportion of individuals who had an idiotype-specific response of the T helper-1 (Th1)-type (IFN- γ - and/or IL-2-secreting cells) [22, 23] was significantly higher in patients with indolent disease (MGUS and MM stage I) compared with those with advanced MM (stage II/III). In contrast, cells secreting the Th2-subtype cytokine profile (IL-4 only) [22, 23] were seen more frequently in patients with advanced MM (stage II/III) [15]. A similar pattern of cytokine secretion was also reported by others [24]. Collectively, these findings indicate that the existing idiotype-specific immune response is too weak to control the growth of myeloma cells *in vivo* and that a shift from an idiotype-specific type-1 response, i.e., Th1 and T cytotoxic-1 (Tc1) [25], in early MM to a type-2 response (Th2 and probably Tc2; [25] in advanced disease may have occurred. These studies provide indirect evidence that idiotype-specific T cells may have a regulatory impact on human tumor B cells. Indeed, our recent study using a myeloma murine model clearly showed that idiotype-specific Th1 and TC1 are cytolytic to myeloma cells, while Th2 cells promote myeloma growth [26].

To examine whether idiotype-specific T cells can recognize and kill myeloma cells, we generated idiotype-specific cytotoxic T lymphocyte (CTL) lines from myeloma patients [27]. To enhance the immunogenicity of idiotype proteins, we used dendritic cells (DCs) as antigen-presenting cells. After repeated rounds of in vitro T-cell stimulation with idiotype-pulsed autologous DCs, idiotype-specific T-cell lines, which consisted of both CD4⁺ and CD8⁺ T cells, were generated and propagated from the peripheral blood mononuclear cells (PBMCs) of myeloma patients. Idiotype-specific proliferative responses were observed when these T cells were rechallenged with the autologous, but not allogeneic, idiotype-pulsed DCs. By using a standard ⁵¹chromium-release assay, our results showed that idiotype-specific CTLs not only recognized and lysed autologous idiotype-pulsed DCs but also significantly killed autologous primary myeloma cells. The cytotoxicity was MHC class I- and, to a lesser extent, class II-restricted, suggesting that myeloma cells could process idiotype protein and present idiotype peptides in the context of their surface MHC molecules. Taken together, these findings provide direct evidence that myeloma plasma cells express idiotype peptides-MHC molecules on their surface and are susceptible to idiotype-specific T cell-mediated lysis.

Idiotype proteins have been used as myeloma antigens for immunotherapies of MM for the past 14 years [3]. Our group at the Karolinska Institutet, Stockholm, Sweden, was the first to introduce active immunization of myeloma patients with Id proteins [28, 29]. In our first pilot study, we recruited and immunized five previously untreated patients with stages I–III MM with the autologous Id protein precipitated in an aluminum phosphate suspension [28]. In three patients, an anti-Id T-cell response amplified 1.9- to 5-fold during the immunization. However, the induced T-cell response was transient and was eliminated during repeated immunization. The disease was stable in all patients, and no side effects or clinical responses were noted. In our second series of the study, immunization was performed by subcutaneous or intradermal injection of Id protein and granulocyte-monocyte colony-stimulating factor (GM-CSF) [29]. Five patients with IgG myeloma were treated, and an Id-specific type-1 T-cell response developed in all of them. One patient had a clinical response, defined by a significant decrease in serum Id protein (from 20 g/L to 7 g/L) and normalization of serum Ig levels. Although these studies involved a limited number of patients, the results clearly indicated that Id protein vaccination, particularly in combination with GM-CSF, was able to induce specific anti-Id cellular and humoral immune responses, which were occasionally accompanied by a clinical response in treated patients.

Other clinical settings for immunotherapy could be minimal residual disease status achieved by high-dose chemotherapy and early host immunologic recovery following stem cell transplantation. These are supported by a study from Massaia and coworkers [30] showing that Id vaccination of myeloma patients with minimal residual disease was able to induce a strong Id-specific cellular immunity in many of the patients. In their study, 12 patients who had been treated with high-dose chemotherapy followed by stem cell support received Id–keyhole limpet hemocyanin (KLH) vaccines and a low dose of GM-CSF or IL-2. Generation of Id-specific T-cell proliferative responses was documented in only two cases; however, a positive, Id-specific, delayed-type hypersensitivity (DTH) skin test reaction was observed in

eight out of the ten patients studied. The induction of humoral and cellular immune responses to KLH was observed in 100% and 80% of the patients, respectively, suggesting that the majority of patients were already able to mount immune responses to KLH shortly after high-dose therapy and stem cell transplantation. Collectively, these results indicate that immunization of myeloma patients with the autologous Id protein, together with GM-CSF, might be a promising method of immunotherapy [31].

Since the renewed interest in using myeloid DCs as tumor vaccine, several groups published their results of idiotype-pulsed DC vaccination studies in MM. Wen and coworkers [19] reported vaccinating an MM patient with autologous Id protein-pulsed DCs generated from blood adherent cells. Enhanced Id-specific cellular and humoral responses were observed in the patient. The immune responses were associated with a transient minor decrease in the serum Id protein level. In their subsequent study, six additional patients were treated according to the same protocol [32]. An immune response against Id was demonstrated in many of the patients. A minor clinical response (25% reduction in the M-component) was observed in one patient and stable disease in the remaining patients. Reichardt and coworkers [33] reported their experience with Id-pulsed DC vaccination in 12 myeloma patients after autologous peripheral blood stem cell transplantation. Their results were less compelling because only 2 out of 12 patients mounted cellular Id-specific proliferative responses as the sole evidence for effective vaccination. Nevertheless, all myeloma patients could mount a strong anti-KLH response despite recent high-dose therapy. Similar results were also obtained in their subsequent study involving 26 patients treated on the same protocol [34]. Although 24 out of 26 patients generated a KLH-specific cellular proliferative immune response, an Id-specific proliferative immune response developed in only four patients. No clinical benefit was observed. These results suggest that DC-based Id vaccination is feasible after transplantation and can induce an Id-specific T-cell response in certain patients.

Other clinical trials of Id-pulsed DC vaccination in myeloma patients have been reported. Cull and coworkers [35] reported on their experience of vaccinating two patients with advanced refractory MM with Id-pulsed DCs combined with GM-CSF. An anti-Id T-cell proliferative response was detected in both patients, which was associated with IFN- γ production by the T cells. One patient also had an anti-Id humoral response. Titzer and coworkers [36] treated 11 patients with advanced MM with Id-pulsed, CD34⁺ stem cell-derived DCs and GM-CSF. After vaccination, three out of ten analyzed patients showed an increased anti-Id antibody titer, and four out of the ten patients had an Id-specific T-cell response measured by ELISPOT assay.

To improve the efficacy of DC vaccination in myeloma, we investigated the use of Id-pulsed mature DCs administered subcutaneously. Five patients with stable partial remission following high-dose chemotherapy were vaccinated at least 4 months posttransplantation [37]. After four DC vaccinations, Id-specific T-cell responses were elicited in four patients and anti-Id B-cell responses in all five patients. A 50% reduction in serum Id protein was observed in one immunologically responding patient and persisted for more than 1 year; stable disease was noted in the other three patients. The remaining patient without an immune response to the

vaccination experienced disease relapse. Similar results were recently reported by Curti and coworkers [38]. In their study, 15 patients received DCs pulsed with Id proteins or their peptides, and an Id-specific IFN- γ response was seen in eight patients. Clinically, 7 out of the 15 patients had stable disease after a median follow-up of 26 months, one patient achieved durable partial remission after 40 months, and seven patients progressed. Alternatively, Id-pulsed allogeneic DCs could also be used to vaccinate myeloma patients [39]. Taken together, these results indicate that subcutaneous DC vaccination indeed induces better antimyeloma responses than intravenous DC vaccination.

Recently we investigated the use of idiotype- and KLH-pulsed, CD40 ligand-matured DCs administered intranodally. Nine patients with smoldering or stable myeloma without treatment were enrolled, and DC vaccines were administered at weekly intervals for a total of four doses. Following vaccination, all patients mounted Id-specific IFN- γ T-cell response. IL-4 response was elicited in two and skin DTH reaction in seven patients. More importantly, idiotype-specific CTL responses were also detected in five patients. Most if not all patients mounted a positive T-cell response to KLH following vaccination. At 1-year follow-up, six of the nine patients had stable disease, while three patients had slowly progressive disease even during the vaccination period. At 5-year follow-up, four of the six patients continued with stable disease. No major side effects were noted. These results suggest that intranodal administration of Id-pulsed CD40 ligand-matured DCs was able to induce idiotype-specific T and B cell and perhaps clinical responses in patients [40]. In line with these results, Lacy and coworkers reported that idiotype-pulsed DCs following autologous transplantation for MM may be associated with prolonged survival [41].

2.3 Novel Antigenic Targets for Immune Targeting

2.3.1 *Dickkopf-1 (DKK1)*

DKK1 is a secreted protein that specifically inhibits the Wnt/ β -catenin signaling by interacting with the co-receptor Lrp-6 [42, 43]. Previous studies have shown that the *DKK1* gene has restricted expression in placenta and mesenchymal stem cells (MSCs) and not in other normal tissues [44, 45]. Recent studies demonstrated that DKK1 in myeloma patients was associated with the presence of lytic bone lesions [46]. Immunohistochemical analysis of bone marrow biopsy specimens showed that only myeloma cells contain detectable DKK1. Recombinant human DKK1 or bone marrow serum containing an elevated level of DKK1 inhibited the differentiation of osteoblast precursor cells in vitro. Furthermore, anti-DKK1 antibody treatment was associated with reduced tumor growth in myeloma mouse models [47–49]. These results indicate that DKK1 is an important player in myeloma bone disease.

The identification of novel tumor-associated antigens, particularly those shared among patients, is urgently needed to improve the efficacy of immunotherapy for MM. For this purpose, we examined whether DKK1 could be a good candidate.

We identified and synthesized DKK1 peptides for HLA-A*0201 and confirmed their immunogenicity by in vivo immunization of HLA-A*0201 transgenic mice. We detected low frequencies of DKK1 peptide-specific CD8⁺ T cells in myeloma patients by using peptide tetramers and generated peptide-specific T-cell lines and clones from HLA-A*0201⁺ blood donors and myeloma patients. These T cells efficiently lysed peptide-pulsed but not unpulsed T2 or autologous DCs, DKK1⁺/HLA-A*0201⁺ myeloma cell lines U266 and IM-9, and more importantly, HLA-A*0201⁺ primary myeloma cells from patients. No killing was observed on DKK1⁺/HLA-A*0201⁻ myeloma cell lines and primary myeloma cells or HLA-A*0201⁺ normal lymphocytes, including B cells [50]. These T cells were also therapeutic in vivo against established myeloma in SCID-hu mice after adoptive transfer. These results indicate that these T cells were potent CTLs and recognized DKK1 peptides naturally presented by myeloma cells in the context of HLA-A*0201 molecules. Hence, our study identified DKK1 as a potentially important antigen for immunotherapy in MM.

Inhibiting DKK1 activity by using specific monoclonal antibodies (mAbs) to treat MM and myeloma-associated bone disease is also a novel approach because DKK1 has been shown to contribute to osteolytic bone disease in MM by inhibiting the differentiation of osteoblasts [46]. A humanized DKK1-neutralizing mAb, BHQ880 has been developed by Novartis and tested in preclinical studies [47–49]. In both murine [48] and xenograft human [47, 49] myeloma mouse models, this mAb was shown to sustain or increase the numbers of osteoblasts, protect myeloma-induced bone loss, and reduce the development of osteolytic bone lesions. Furthermore, the mAb was also shown to inhibit the growth of xenografted human myeloma cells in SCID-hu [47] or SCID-rab [49] mouse models. These results provide the rationale for clinical evaluation of BHQ880 to improve bone disease and to inhibit myeloma growth.

2.3.2 β_2 -Microglobulin (β_2 M)

β_2 M is an 11.6-kDa non-glycosylated polypeptide composed of 100 amino acids. It is part of the MHC class I molecule on the cell surface of nucleated cells. Its best characterized function is to interact with and stabilize the tertiary structure of the MHC class I α -chain [51]. Because it is non-covalently associated with the α -chain and has no direct attachment to the cell membrane, β_2 M on the cell surface can exchange with free β_2 M present in serum-containing medium [52]. Free β_2 M is found in body fluids under physiological conditions as a result of intracellular release. Elevated levels of serum β_2 M are present in hematological malignancies, including lymphomas [53], leukemias [54, 55], and MM [56, 57] and correlate with a poor prognosis regardless of a patient's renal function [57, 58]. This observation suggests an important, yet unidentified, role of this protein in these malignancies.

While examining the effects of β_2 M on myeloma cells, we made a novel and exciting discovery, namely, that mAbs against β_2 M have a remarkably strong apoptotic effect on myeloma cells and on other hematological tumor cells [59]. Anti- β_2 M mAbs induced apoptosis in up to 90% of cells in a 48-h culture in all tested human myeloma cell lines ($n=8$) and primary myeloma cells from patients ($n=10$). The mAbs also kill β_2 M/MHC class I-bearing lymphoma and leukemia cells. Anti-MHC class I mAbs (LY5.1, IgG1 or W6/32, IgG2a), purified mouse IgG and IgG1 had no effect. Cell death occurred rapidly, without the need for exogenous immunological effector mechanisms (e.g., complement or NK cells) or secondary cross-linking. Anti- β_2 M mAb-induced apoptosis in myeloma cells was not blocked by soluble β_2 M (10–100 μ g/mL, 3- to 30-fold higher than the levels in most MM patients), IL-6, or other myeloma growth and survival factors and was stronger than apoptosis observed with chemotherapy drugs currently used to treat MM (e.g., dexamethasone).

Although the expression of β_2 M on normal hematopoietic cells is a potential safety concern, the mAbs were selective to tumor-transformed cells and did not induce apoptosis of normal cells, including T and B lymphocytes, plasma cells, and purified CD34⁺ stem cells. Furthermore, the mAbs selectively and effectively killed myeloma cells without damaging osteoclasts (OCs) or PBMCs in their cocultures with myeloma cells. More importantly, anti- β_2 M mAbs are therapeutic in vivo in xenograft SCID and SCID-hu mouse models [59], and in the HLA-A2-transgenic NOD-SCID (A2-NOD-SCID) models of myeloma, in which every mouse tissue expresses human MHC class I/ β_2 M molecules and circulating human β_2 M could reach the levels seen in most myeloma patients without causing damage to normal human hematopoiesis or murine organs [60]. Interestingly, following our publication, others have reported similar results using anti-MHC class single-chain Fv diabody or anti- β_2 M antibodies, respectively, in human myeloma [61], renal cell carcinoma [62], and prostate cancer [63]. Therefore, such mAbs offer the potential for a therapeutic approach to hematological malignancies.

The mAbs induced apoptosis in myeloma cells by recruiting MHC class I to lipid rafts, activated JNK, and inhibited PI3K/Akt and ERK pathways [59]. Growth and survival cytokines such as IL-6 and IGF-I, which could protect myeloma cells from dexamethasone-induced apoptosis, did not affect mAb-mediated cell death. We elucidated the mechanisms underlying anti- β_2 M mAb-induced PI3K/Akt and ERK inhibition and the inability of IL-6 and IGF-I to protect myeloma cells from mAb-induced apoptosis. We focused on lipid rafts and confirmed that these membrane microdomains are required for IL-6 and IGF-I signaling. By recruiting MHC class I into lipid rafts, anti- β_2 M mAbs excluded IL-6 and IGF-I receptors and their substrates from the rafts. The mAbs were not only redistributed to the receptors in cell membrane, but also abrogated IL-6- or IGF-I-mediated JAK/STAT3, PI3K/Akt, and Ras/Raf/ERK pathway signaling, which are otherwise constitutively activated in myeloma cells [64]. Thus, our study further defines the tumoricidal mechanism of the mAbs and provides strong evidence to support the potential of these mAbs as therapeutic agents for myeloma.

2.3.3 CS1

CS1, a glycoprotein and a member of the immunoglobulin gene superfamily, has been found to be highly expressed on tumor cells from myeloma patients, and soluble serum CS1 correlates with active disease in myeloma patients [65]. However, CS1 is also expressed by NK cells, NKT cells, and CD8⁺ T cells [65].

As the above data suggest that CS1 could be a novel target for therapy, a humanized mAb against CS1, HuLuc63, was generated [65]. HuLuc63 inhibited myeloma cell binding to bone marrow stromal cells and induced antibody-dependent cell-mediated cytotoxicity (ADCC) against myeloma cells in dose-dependent and CS1-specific manners. Furthermore, the mAb mediated autologous ADCC against primary myeloma cells resistant to conventional or novel therapies, and pretreatment with conventional or novel antimyeloma drugs markedly enhanced HuLuc63-induced myeloma cell lysis. In vivo injection of the mAb significantly induced tumor regression in xenograft myeloma mouse models [66]. In addition, a recent study showed that HuLuc63 (elotuzumab) in combination with bortezomib exhibited significantly enhanced in vivo antimyeloma activity in human myeloma-xenografted mouse model [67]. Based on these results, phase-I clinical trials are underway to evaluate the safety and toxicity of the mAb in myeloma patients.

2.3.4 C-Reactive Protein

C-reactive protein (CRP), the first acute-phase protein described and an ancient and highly conserved protein of the pentraxin family, has five identical subunits forming a planar ring that confers very high stability to the protein. In healthy young adults, the median concentration of CRP is 0.8 mg/L, but following an acute-phase stimulus, values may increase by 10,000-fold, from less than 50 µg/L to more than 500 mg/L [68, 69]. Plasma CRP is produced primarily in the liver, synthesized by hepatocytes in response to intermediary inflammatory cytokines such as IL-1 and IL-6. CRP has been shown to bind to a variety of ligands, including pneumococcal polysaccharides, membrane phospholipids, apoptotic cells, fibronectin, and ribonuclear particles [69]. CRP also binds C1q and activates the classical complement cascade and binds Fcγ receptors (FcγRs) leading to indirect (via classical complement) and direct opsonization (via FcγRs) [69]. Through these mechanisms, CRP can play a direct role in a wide range of inflammatory processes and contributes to innate host immunity.

CRP is a sensitive systemic marker of inflammation and tissue damage. Elevated levels of CRP are present in patients with infections, inflammatory diseases, necrosis such as myocardial infarction [70], or malignancies including MM [71, 72], lymphoma [73, 74], and carcinoma [75]. Accumulating evidence has strongly suggested that in cardiovascular disease CRP is not only a marker of inflammation but also contributes to pathogenesis of the disease [76]. Evidence includes the results that CRP directly activated various vascular cells to secrete cytokines, enhanced their expression of adhesion molecules, increased monocyte/macrophage chemotaxis and

adhesion, facilitated extracellular matrix remodeling, enhanced endothelial dysfunction, and activated coagulation [77, 78]. Furthermore, human CRP has been shown to increase myocardial and cerebral infarct size in rats subjected to coronary or cerebral artery ligation, respectively, and this drastic enhancement of infarct size by human CRP was completely abrogated by in vivo complement depletion of the rats using cobra venom factor [79, 80].

These findings led to our hypothesis that CRP may also have a functional role in tumor cells since elevated levels of CRP are present in cancer patients [71–74]. We discovered that addition of CRP to cultures at levels seen in patients with MM or other tumors promoted myeloma cell proliferation under stressed conditions and protected myeloma cells from chemotherapy drug-, IL-6 withdrawal-, or serum deprivation-induced apoptosis in vitro. The protective effect was verified in vivo in myeloma SCID and SCID-hu mouse models. These phenomena may be clinically relevant since CRP was found accumulating on the surface of bone marrow myeloma cells from patients with MM. Although myeloma cells expressed all three types of FcγR, we identified FcγRII, more specifically, FcγRIIA and FcγRIIC as the primary receptors for CRP on the tumor cells. Our results demonstrated that CRP activated PI3K/Akt, ERK, and NF-κB in treated cells via binding to these receptors, which led to inhibited activation of caspase cascades induced by chemotherapy drugs such as dexamethasone and undermined the therapeutic efficacy of chemotherapy in the myeloma mouse models [81]. Thus, our study demonstrates that CRP plays an active role in regulating tumor cell growth and survival and suggests that targeting CRP by CRP-neutralizing antibodies or FcγRII-blocking antibodies may sensitize myeloma cells to chemotherapy drug-induced apoptosis.

2.3.5 Cancer-Testis Antigens

Numerous studies have shown that the Cancer-Testis (CT) antigens, such as MAGE-A3 and NY-ESO-1, may be expressed by myeloma cells [82–84]. DNA microarray analysis of gene expression of >95% pure myeloma cells from more than 300 patients showed that the genes of these antigens were expressed in the tumor cells, particularly from patients with relapsed disease or abnormal cytogenetics (in 7–20% of MGUS and newly diagnosed MM and in 40–50% of relapsed patients or in patients with cytogenetic abnormalities) [85, 86]. With the use of specific mAbs against MAGE-A3 or NY-ESO-1, it was evident that the proteins of these antigens were also expressed in the tumor cells of patients with positive gene expression. Moreover, cellular immune responses against MAGE-C1/CT7 and humoral responses against other CT antigens, such as MAGE-A1 and SSX-1, can be detected in MM patients [87].

Recent studies indicated that the expression of CT antigens on myeloma cells may represent a predictor of outcome of myeloma patients. Among CT antigens examined, MAGE-C1/CT-7 is the most prevalent CT antigen, expressed in about 60% of myeloma cells of patients [88, 89]. This CT antigen was more frequently expressed

in myeloma cells with an elevated proliferation rate compared with myeloma cells with a low proliferation rate and correlated well with overall survival [89, 90]. In another study, the expression of MAGE-C1 gene represented an important indicator of early relapse and dramatically reduced survival of patients after allogeneic stem cell transplantation [91].

Van Rhee and his colleague reported their study of immunization of a sibling donor with recombinant CT protein for allogeneic/syngeneic transplantation [92]. As MAGE-A3 is frequently expressed in high-risk MM, they immunized a healthy donor with MAGE-A3 protein formulated in AS02B to transfer immunity to her identical twin, diagnosed with MAGE-A3-positive MM. After a melphalan 200 mg/m syngeneic peripheral blood stem cell transplant, primed donor cells collected after immunizations were transferred and followed by repeated patient immunizations. Strong MAGE-A3-specific antibody, CTL, and T-helper responses were induced in both twins. A humoral response was transferred to the patient with the donor peripheral blood stem cells and increased by booster immunization. The CTL response targeted a previously undescribed HLA-A*6801 binding MAGE-A3115-123 peptide. MAGE-A3115-123 CTLs were detected in the patient more than 1 year after the last immunization. Multiple T-helper cellular responses were detected with the dominant response to an HLA-DR11-restricted MAGE-A3 epitope. The patient remained in remission 2.5 years after the second transplant. These results show that immunization of a healthy donor with a defined cancer-testis protein can induce immune responses that can be transferred and expanded posttransplant in the recipient.

2.3.6 Other Potential Targets

Another potential target is CD40, which is expressed on B-cell tumors including MM. Two humanized anti-CD40 mAbs, SGN-40 and HCD122, have been developed and tested in preclinical studies [93, 94]. These mAb induced modest cytotoxicity in myeloma cell lines and primary myeloma cells from patients, but can effectively kill myeloma cell via mediating ADCC. Further, the immunomodulatory drug lenalidomide further augmented anti-CD40 mAb-induced cytotoxicity in human myeloma cells [95]. In addition to anti-CD40 mAbs, other mAbs currently in clinical trials include anti-CD74, anti-CD56, and anti-HM1.24 [96].

Furthermore, other antigens, such as MUC-1 [97–99], sperm protein 17 (Sp17) [100, 101], and HM1.24 [102–104], may also be expressed on myeloma cells, and MHC-restricted antigens MUC-1 [105] and Sp17 [106]-specific CTLs have been generated from myeloma patients that were able to lyse myeloma cells. Recently, a phase-I/II clinical trial has been initiated to examine the safety and efficacy of Sp17-pulsed DC vaccination in myeloma patients [100]. However, there is evidence that Sp17 is also expressed on normal T and B cells [107]; hence, although these antigens may be potential targets, further research is warranted to examine their applicability for immunotherapy in MM.

2.4 Conclusion

Immunotherapy has become an important part of therapeutic strategies for hematological malignancies including MM. Passive immunotherapies using mAbs directed against tumor-associated surface antigens, such as CD20 (rituximab, Rituxan), CD22 (epratuzumab, LymphoCide), CD52 (alemtuzumab, Campath), and major histocompatibility complex (MHC) class II (Hu1D10, Remitogen), have been approved by the US Food and Drug Administration and are in widespread use either alone or in combination with chemotherapy or with other biological agents. These reagents can be applied as conjugates with toxins or isotopes as means to deliver a toxic compound or radioactivity to tumor cells, or as unlabeled antibodies to cause direct anticancer effects or induce a secondary immune response against tumor cells via a number of mechanisms. Thus far, encouraging results have been obtained in the treatment of various hematological malignancies, including non-Hodgkin's lymphomas, chronic lymphocytic leukemia, Waldenström's macroglobulinemia, and MM [108–110]. Active immunotherapy, in which the patients are induced to generate a specific immune response against the tumor cells, has long been a goal of tumor immunologists. Idiotypic proteins have been used as the only tumor antigen for clinical immunotherapies for the past 14 years. Although tumor-specific, idiotype proteins are weak tumor antigen and need to be prepared from each patient [111]. Idiotype-based vaccines have been shown to induce or enhance idiotype-specific immunity, indicating that the vaccines are able to elicit a specific immune response [112]. However, clinical response is still a rare event, occurring only in a minority of treated patients, suggesting that the elicited or enhanced immunity is still too weak to cause significant tumor destruction. Thus far, although no active immunotherapy maneuver has yet proven to be effective in the clinic, intensive efforts are underway to develop such an approach. Experiments in animal models have shown that vaccination against actively growing tumors is much more difficult to accomplish [113, 114]. It is therefore not surprising that clinical trials in patients with gross disease will be the most difficult setting in which to demonstrate efficacy. Thus, it is conceivable that immunotherapy may work better in patients in remission or with minimal residual disease, who are more likely to be able to generate a robust immune response against the tumor and to derive therapeutic benefit. Nevertheless, with a better understanding of the immune system and tumor microenvironment, as well as identification and development of many novel targets and methods for immune targeting, there is a realistic hope that immunotherapies will soon be a part of conventional treatment modalities in MM and help control or even cure the disease.

Conflict-of-interest disclosure: The author declares no competing financial interests.

Acknowledgements This work was supported by institutional start-up funds from the University of Texas M. D. Anderson Cancer Center, the Center for Targeted Therapy of The University of Texas M. D. Anderson Cancer Center, grants from the National Cancer Institute (R01 CA96569, R01 CA103978, and R01 CA138402), the Leukemia & Lymphoma Society, Multiple Myeloma Research Foundation, and Commonwealth Foundation for Cancer Research. I thank Mrs. Kimberly Jensen for providing editorial assistance.

References

1. Kyle RA, Vincent Rajkumar S (2006) Treatment of multiple myeloma: an emphasis on new developments. *Ann Med* 38:111–115
2. Anderson KC (2007) Targeted therapy of multiple myeloma based upon tumor-microenvironmental interactions. *Exp Hematol* 35:155–162
3. Yi Q (2009) Novel immunotherapies. *Cancer J* 15:502–510
4. Tricot G, Vesole DH, Jagannath S, Hilton J, Munshi N, Barlogie B (1996) Graft-versus-myeloma effect: proof of principle. *Blood* 87:1196–1198
5. Verdonck LF, Lokhorst HM, Dekker AW, Nieuwenhuis HK, Petersen EJ (1996) Graft-versus-myeloma effect in two cases. *Lancet* 347:800–801
6. Lokhorst HM, Wu K, Verdonck LF, Laterveer LL, van de Donk NW, van Oers MH, Cornelissen JJ, Schattenberg AV (2004) The occurrence of graft-versus-host disease is the major predictive factor for response to donor lymphocyte infusions in multiple myeloma. *Blood* 103:4362–4364
7. Campbell MJ, Esserman L, Byars NE, Allison AC, Levy R (1990) Idiotypic vaccination against murine B cell lymphoma. Humoral and cellular requirements for the full expression of antitumor immunity. *J Immunol* 145:1029–1036
8. Kaminski MS, Kitamura K, Maloney DG, Levy R (1987) Idiotypic vaccination against murine B cell lymphoma. Inhibition of tumor immunity by free idiotype protein. *J Immunol* 138:1289–1296
9. King CA, Spellerberg MB, Zhu D, Rice J, Sahota SS, Thompson AR, Hamblin TJ, Radl J, Stevenson FK (1998) DNA vaccines with single-chain Fv fused to fragment C of tetanus toxin induce protective immunity against lymphoma and myeloma. *Nat Med* 4:1281–1286
10. Sirisinha S, Eisen HN (1971) Autoimmune-like antibodies to the ligand-binding sites of myeloma proteins. *Proc Natl Acad Sci USA* 68:3130–3135
11. Wang S, Hong S, Wezeman M, Qian J, Yang J, Yi Q (2007) Dendritic cell vaccine but not idiotype-KLH protein vaccine primes therapeutic tumor-specific immunity against multiple myeloma. *Front Biosci* 12:3566–3575
12. Holm G, Bergenbrant S, Lefvert AK, Yi Q, Osterborg A, Mellstedt H (1991) Anti-idiotypic immunity as a potential regulator in myeloma and related diseases. *Ann N Y Acad Sci* 636:178–183
13. Osterborg A, Yi Q, Bergenbrant S, Holm G, Lefvert AK, Mellstedt H (1995) Idiotypic-specific T cells in multiple myeloma stage I: an evaluation by four different functional tests. *Br J Haematol* 89:110–116
14. Yi Q, Bergenbrant S, Osterborg A, Osby E, Ostman R, Bjorkholm M, Holm G, Lefvert AK (1993) T-cell stimulation induced by idiotypes on monoclonal immunoglobulins in patients with monoclonal gammopathies. *Scand J Immunol* 38:529–534
15. Yi Q, Osterborg A, Bergenbrant S, Mellstedt H, Holm G, Lefvert AK (1995) Idiotypic-reactive T-cell subsets and tumor load in monoclonal gammopathies. *Blood* 86:3043–3049
16. Fagerberg J, Yi Q, Gigliotti D, Harmenberg U, Ruden U, Persson B, Osterborg A, Mellstedt H (1999) T-cell-epitope mapping of the idiotypic monoclonal IgG heavy and light chains in multiple myeloma. *Int J Cancer* 80:671–680
17. Hansson L, Rabbani H, Fagerberg J, Osterborg A, Mellstedt H (2003) T-cell epitopes within the complementarity-determining and framework regions of the tumor-derived immunoglobulin heavy chain in multiple myeloma. *Blood* 101:4930–4936
18. Szea DM, Brown RD, Yang S, Gibson J, Ho J, de St Groth BF, Basten A, Joshua DE (2003) Prediction of high affinity class I-restricted multiple myeloma idiotype peptide epitopes. *Leuk Lymphoma* 44:1557–1568
19. Wen YJ, Ling M, Bailey-Wood R, Lim SH (1998) Idiotypic protein-pulsed adherent peripheral blood mononuclear cell-derived dendritic cells prime immune system in multiple myeloma. *Clin Cancer Res* 4:957–962

20. Dabadghao S, Bergenbrant S, Anton D, He W, Holm G, Yi Q (1998) Anti-idiotypic T-cell activation in multiple myeloma induced by M-component fragments presented by dendritic cells. *Br J Haematol* 100:647–654
21. Yi Q, Eriksson I, He W, Holm G, Mellstedt H, Osterborg A (1997) Idiotypic-specific T lymphocytes in monoclonal gammopathies: evidence for the presence of CD4+ and CD8+ subsets. *Br J Haematol* 96:338–345
22. Romagnani S (1991) Human TH1 and TH2 subsets: doubt no more. *Immunol Today* 12:256–257
23. Romagnani S (1992) Human TH1 and TH2 subsets: regulation of differentiation and role in protection and immunopathology. *Int Arch Allergy Immunol* 98:279–285
24. Walchner M, Wick M (1997) Elevation of CD8+ CD11b+ Leu-8- T cells is associated with the humoral immunodeficiency in myeloma patients. *Clin Exp Immunol* 109:310–316
25. Salgame P, Abrams JS, Clayberger C, Goldstein H, Convit J, Modlin RL, Bloom BR (1991) Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 254:279–282
26. Hong S, Qian J, Yang J, Li H, Kwak LW, Yi Q (2008) Roles of idiotype-specific t cells in myeloma cell growth and survival: Th1 and CTL cells are tumoricidal while Th2 cells promote tumor growth. *Cancer Res* 68:8456–8464
27. Wen YJ, Barlogie B, Yi Q (2001) Idiotypic-specific cytotoxic T lymphocytes in multiple myeloma: evidence for their capacity to lyse autologous primary tumor cells. *Blood* 97:1750–1755
28. Bergenbrant S, Yi Q, Osterborg A, Bjorkholm M, Osby E, Mellstedt H, Lefvert AK, Holm G (1996) Modulation of anti-idiotypic immune response by immunization with the autologous M-component protein in multiple myeloma patients. *Br J Haematol* 92:840–846
29. Osterborg A, Yi Q, Henriksson L, Fagerberg J, Bergenbrant S, Jeddi-Tehrani M, Ruden U, Lefvert AK, Holm G, Mellstedt H (1998) Idiotypic immunization combined with granulocyte-macrophage colony-stimulating factor in myeloma patients induced type I, major histocompatibility complex-restricted, CD8- and CD4-specific T-cell responses. *Blood* 91:2459–2466
30. Massaia M, Borriero P, Battaglio S, Mariani S, Beggiato E, Napoli P, Voena C, Bianchi A, Coscia M, Besostri B, Peola S, Stiefel T, Even J, Novero D, Boccadoro M, Pileri A (1999) Idiotypic vaccination in human myeloma: generation of tumor-specific immune responses after high-dose chemotherapy. *Blood* 94:673–683
31. Coscia M, Mariani S, Battaglio S, Di Bello C, Fiore F, Foglietta M, Pileri A, Boccadoro M, Massaia M (2004) Long-term follow-up of idiotype vaccination in human myeloma as a maintenance therapy after high-dose chemotherapy. *Leukemia* 18:139–145
32. Lim SH, Bailey-Wood R (1999) Idiotypic protein-pulsed dendritic cell vaccination in multiple myeloma. *Int J Cancer* 83:215–222
33. Reichardt VL, Okada CY, Liso A, Benike CJ, Stockerl-Goldstein KE, Engleman EG, Blume KG, Levy R (1999) Idiotypic vaccination using dendritic cells after autologous peripheral blood stem cell transplantation for multiple myeloma—a feasibility study. *Blood* 93:2411–2419
34. Liso A, Stockerl-Goldstein KE, Auffermann-Gretzinger S, Benike CJ, Reichardt V, van Beckhoven A, Rajapaksa R, Engleman EG, Blume KG, Levy R (2000) Idiotypic vaccination using dendritic cells after autologous peripheral blood progenitor cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant* 6:621–627
35. Cull G, Durrant L, Stainer C, Haynes A, Russell N (1999) Generation of anti-idiotypic immune responses following vaccination with idiotype-protein pulsed dendritic cells in myeloma. *Br J Haematol* 107:648–655
36. Titzer S, Christensen O, Manzke O, Tesch H, Wolf J, Emmerich B, Carsten C, Diehl V, Bohlen H (2000) Vaccination of multiple myeloma patients with idiotype-pulsed dendritic cells: immunological and clinical aspects. *Br J Haematol* 108:805–816
37. Yi Q, Desikan R, Barlogie B, Munshi N (2002) Optimizing dendritic cell-based immunotherapy in multiple myeloma. *Br J Haematol* 117:297–305

38. Curti A, Tosi P, Comoli P, Terragna C, Ferri E, Cellini C, Massaia M, D'Addio A, Giudice V, Di Bello C, Cavo M, Conte R, Gugliotta G, Baccarani M, Lemoli RM (2007) Phase I/II clinical trial of sequential subcutaneous and intravenous delivery of dendritic cell vaccination for refractory multiple myeloma using patient-specific tumour idiotype protein or idiotype (VDJ)-derived class I-restricted peptides. *Br J Haematol* 139:415–424
39. Bendandi M, Rodriguez-Calvillo M, Inoges S, Lopez-Diaz de Cerio A, Perez-Simon JA, Rodriguez-Caballero A, Garcia-Montero A, Almeida J, Zabalegui N, Giraldo P, San Miguel J, Orfao A (2006) Combined vaccination with idiotype-pulsed allogeneic dendritic cells and soluble protein idiotype for multiple myeloma patients relapsing after reduced-intensity conditioning allogeneic stem cell transplantation. *Leuk Lymphoma* 47:29–37
40. Yi Q, Szmania S, Freeman J, Qian J, Rosen NA, Viswamitra S, Cottler-Fox M, Barlogie B, Tricot G, van Rhee F (2010) Optimizing dendritic cell-based immunotherapy in multiple myeloma: intranodal injections of idiotype-pulsed CD40 ligand-matured vaccines led to induction of type-1 and cytotoxic T-cell immune responses in patients. *Br J Haematol* 150(5):554–564
41. Lacy MQ, Mandrekas S, Dispenzieri A, Hayman S, Kumar S, Buadi F, Dingli D, Litzow M, Wettstein P, Padley D, Kabat B, Gastineau D, Rajkumar SV, Gertz MA (2009) Idiotype-pulsed antigen-presenting cells following autologous transplantation for multiple myeloma may be associated with prolonged survival. *Am J Hematol* 84:799–802
42. Mao B, Wu W, Li Y, Hoppe D, Stannek P, Glinka A, Niehrs C (2001) LDL-receptor-related protein 6 is a receptor for Dickkopf proteins. *Nature* 411:321–325
43. Zorn AM (2001) Wnt signalling: antagonistic Dickkopfs. *Curr Biol* 11:R592–R595
44. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391:357–362
45. Gregory CA, Singh H, Perry AS, Prockop DJ (2003) The Wnt signaling inhibitor dickkopf-1 is required for reentry into the cell cycle of human adult stem cells from bone marrow. *J Biol Chem* 278:28067–28078
46. Tian E, Zhan F, Walker R, Rasmussen E, Ma Y, Barlogie B, Shaughnessy JD Jr (2003) The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 349:2483–2494
47. Fulciniti M, Tassone P, Hideshima T, Vallet S, Nanjappa P, Ettenberg SA, Shen Z, Patel N, Tai YT, Chauhan D, Mitsiades C, Prabhala R, Raje N, Anderson KC, Stover DR, Munshi NC (2009) Anti-DKK1 mAb (BHQ880) as a potential therapeutic agent for multiple myeloma. *Blood* 114:371–379
48. Heath DJ, Chantry AD, Buckle CH, Coulton L, Shaughnessy JD Jr, Evans HR, Snowden JA, Stover DR, Vanderkerken K, Croucher PI (2009) Inhibiting Dickkopf-1 (Dkk1) removes suppression of bone formation and prevents the development of osteolytic bone disease in multiple myeloma. *J Bone Miner Res* 24:425–436
49. Yaccoby S, Ling W, Zhan F, Walker R, Barlogie B, Shaughnessy JD Jr (2007) Antibody-based inhibition of DKK1 suppresses tumor-induced bone resorption and multiple myeloma growth in vivo. *Blood* 109:2106–2111
50. Qian J, Xie J, Hong S, Yang J, Zhang L, Han X, Wang M, Zhan F, Shaughnessy JD Jr, Epstein J, Kwak LW, Yi Q (2007) Dickkopf-1 (DKK1) is a widely expressed and potent tumor-associated antigen in multiple myeloma. *Blood* 110:1587–1594
51. Bjorkman PJ, Burmeister WP (1994) Structures of two classes of MHC molecules elucidated: crucial differences and similarities. *Curr Opin Struct Biol* 4:852–856
52. Strominger JL (2002) Human histocompatibility proteins. *Immunol Rev* 185:69–77
53. Cooper EH, Plesner T (1980) Beta-2-microglobulin review: its relevance in clinical oncology. *Med Pediatr Oncol* 8:323–334
54. Molica S, Levato D, Cascavilla N, Levato L, Musto P (1999) Clinico-prognostic implications of simultaneous increased serum levels of soluble CD23 and beta2-microglobulin in B-cell chronic lymphocytic leukemia. *Eur J Haematol* 62:117–122
55. Shvidel L, Hofstein R, Berrebi A (1996) Serum beta-2 microglobulin as a marker of B-cell activation in chronic lymphoid malignancies. *Am J Hematol* 53:148–149

56. Barlogie B, Jagannath S, Desikan KR, Mattox S, Vesole D, Siegel D, Tricot G, Munshi N, Fassas A, Singhal S, Mehta J, Anaissie E, Dhodapkar D, Naucke S, Cromer J, Sawyer J, Epstein J, Spoon D, Ayers D, Cheson B, Crowley J (1999) Total therapy with tandem transplants for newly diagnosed multiple myeloma. *Blood* 93:55–65
57. Bataille R, Durie BG, Grenier J (1983) Serum beta2 microglobulin and survival duration in multiple myeloma: a simple reliable marker for staging. *Br J Haematol* 55:439–447
58. Alexanian R, Barlogie B, Fritsche H (1985) Beta 2 microglobulin in multiple myeloma. *Am J Hematol* 20:345–351
59. Yang J, Qian J, Wezeman M, Wang S, Lin P, Wang M, Yaccoby S, Kwak LW, Barlogie B, Yi Q (2006) Targeting beta(2)-microglobulin for induction of tumor apoptosis in human hematological malignancies. *Cancer Cell* 10:295–307
60. Yang J, Cao Y, Hong S, Li H, Qian J, Kwak LW, Yi Q (2009) Human-like mouse models for testing the efficacy and safety of anti-beta2-microglobulin monoclonal antibodies to treat myeloma. *Clin Cancer Res* 15:951–959
61. Sekimoto E, Ozaki S, Ohshima T, Shibata H, Hashimoto T, Abe M, Kimura N, Hattori K, Kawai S, Kinoshita Y, Yamada-Okabe H, Tsuchiya M, Matsumoto T (2007) A single-chain Fv diabody against human leukocyte antigen-A molecules specifically induces myeloma cell death in the bone marrow environment. *Cancer Res* 67:1184–1192
62. Nomura T, Huang WC, Seo S, Zhou HE, Mimata H, Chung LW (2007) Targeting beta2-microglobulin mediated signaling as a novel therapeutic approach for human renal cell carcinoma. *J Urol* 178:292–300
63. Huang WC, Wu D, Xie Z, Zhou HE, Nomura T, Zayzafoon M, Pohl J, Hsieh CL, Weitzmann MN, Farach-Carson MC, Chung LW (2006) Beta2-microglobulin is a signaling and growth-promoting factor for human prostate cancer bone metastasis. *Cancer Res* 66:9108–9116
64. Yang J, Zhang X, Wang J, Qian J, Zhang L, Wang M, Kwak LW, Yi Q (2007) Anti beta2-microglobulin monoclonal antibodies induce apoptosis in myeloma cells by recruiting MHC class I to and excluding growth and survival cytokine receptors from lipid rafts. *Blood* 110:3028–3035
65. Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, Shum BP, Huseni M, Powers D, Nanisetti A, Zhang Y, Rice AG, van Abbema A, Wong M, Liu G, Zhan F, Dillon M, Chen S, Rhodes S, Fuh F, Tsurushita N, Kumar S, Vexler V, Shaughnessy JD Jr, Barlogie B, van Rhee F, Hussein M, Afar DE, Williams MB (2008) CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. *Clin Cancer Res* 14:2775–2784
66. Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, Lee AI, Podar K, Hideshima T, Rice AG, van Abbema A, Jesaitis L, Caras I, Law D, Weller E, Xie W, Richardson P, Munshi NC, Mathiot C, Avet-Loiseau H, Afar DE, Anderson KC (2008) Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. *Blood* 112:1329–1337
67. van Rhee F, Szmania SM, Dillon M, van Abbema AM, Li X, Stone MK, Garg TK, Shi J, Moreno-Bost AM, Yun R, Balasa B, Ganguly B, Chao D, Rice AG, Zhan F, Shaughnessy JD Jr, Barlogie B, Yaccoby S, Afar DE (2009) Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma. *Mol Cancer Ther* 8:2616–2624
68. Pepys MB, Hirschfield GM (2003) C-reactive protein: a critical update. *J Clin Invest* 111:1805–1812
69. Stein MP, Edberg JC, Kimberly RP, Mangan EK, Bharadwaj D, Mold C, Du Clos TW (2000) C-reactive protein binding to FcγRIIIa on human monocytes and neutrophils is allele-specific. *J Clin Invest* 105:369–376
70. Pepys MB (1983) C-reactive protein: the role of an ancient protein in modern rheumatology. *Clin Exp Rheumatol* 1:3–7
71. Bataille R, Boccadoro M, Klein B, Durie B, Pileri A (1992) C-reactive protein and beta-2 microglobulin produce a simple and powerful myeloma staging system. *Blood* 80:733–737
72. Tienhaara A, Pulkki K, Mattila K, Irtala K, Pelliniemi TT (1994) Serum immunoreactive interleukin-6 and C-reactive protein levels in patients with multiple myeloma at diagnosis. *Br J Haematol* 86:391–393

73. Legouffe E, Rodriguez C, Picot MC, Richard B, Klein B, Rossi JF, Commes T (1998) C-reactive protein serum level is a valuable and simple prognostic marker in non Hodgkin's lymphoma. *Leuk Lymphoma* 31:351–357
74. Pedersen LM, Bergmann OJ (2003) Urinary albumin excretion and its relationship to C-reactive protein and proinflammatory cytokines in patients with cancer and febrile neutropenia. *Scand J Infect Dis* 35:491–494
75. Reichle A, Bross K, Vogt T, Bataille F, Wild P, Berand A, Krause SW, Andreessen R (2004) Pioglitazone and rofecoxib combined with angiostatically scheduled trofosfamide in the treatment of far-advanced melanoma and soft tissue sarcoma. *Cancer* 101:2247–2256
76. Venugopal SK, Devaraj S, Jialal I (2005) Effect of C-reactive protein on vascular cells: evidence for a proinflammatory, proatherogenic role. *Curr Opin Nephrol Hypertens* 14:33–37
77. Berenson JR, Yang HH, Sadler K, Jarutirasarn SG, Vescio RA, Mapes R, Purner M, Lee SP, Wilson J, Morrison B, Adams J, Schenkein D, Swift R (2006) Phase I/II trial assessing bortezomib and melphalan combination therapy for the treatment of patients with relapsed or refractory multiple myeloma. *J Clin Oncol* 24:937–944
78. Garcia F, Sepulveda P, Liegeard P, Gregoire J, Hermann E, Lemonnier F, Langlade-Demoyen P, Hontebeyrie M, Lone YC (2003) Identification of HLA-A*0201-restricted cytotoxic T-cell epitopes of *Trypanosoma cruzi* TcP2beta protein in HLA-transgenic mice and patients. *Microbes Infect* 5:351–359
79. Gill R, Kemp JA, Sabin C, Pepys MB (2004) Human C-reactive protein increases cerebral infarct size after middle cerebral artery occlusion in adult rats. *J Cereb Blood Flow Metab* 24:1214–1218
80. Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, Pepys MB (1999) C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med* 190:1733–1740
81. Yang J, Wezeman M, Zhang X, Lin P, Wang M, Qian J, Wan B, Kwak LW, Yu L, Yi Q (2007) Human C-reactive protein binds activating fcgamma receptors and protects myeloma tumor cells from apoptosis. *Cancer Cell* 12:252–265
82. Dhodapkar MV, Osman K, Teruya-Feldstein J, Filippa D, Hedvat CV, Iversen K, Kolb D, Geller MD, Hassoun H, Kewalramani T, Comenzo RL, Coplan K, Chen YT, Jungbluth AA (2003) Expression of cancer/testis (CT) antigens MAGE-A1, MAGE-A3, MAGE-A4, CT-7, and NY-ESO-1 in malignant gammopathies is heterogeneous and correlates with site, stage and risk status of disease. *Cancer Immun* 3:9
83. Pellat-Deceunynck C, Mellerin MP, Labarriere N, Jego G, Moreau-Aubry A, Harousseau JL, Jotereau F, Bataille R (2000) The cancer germ-line genes MAGE-1, MAGE-3 and PRAME are commonly expressed by human myeloma cells. *Eur J Immunol* 30:803–809
84. van Baren N, Brasseur F, Godelaine D, Hames G, Ferrant A, Lehmann F, Andre M, Ravoet C, Doyen C, Spagnoli GC, Bakkus M, Thielemans K, Boon T (1999) Genes encoding tumor-specific antigens are expressed in human myeloma cells. *Blood* 94:1156–1164
85. Gupta SK, Pei L, Drooijenbroeck JV, Szmania SM, Yacobby S, Batchu RB, Spagnoli GC, Tricot G, Epstein J, van Rhee F (2002) Intra- and intertumoral variation in the expression of cancer testis antigens, MAGE-3 and NY-ESO-1 in multiple myeloma. *Blood* 100:603a
86. Gupta SK, Shaughnessy J, Drooijenbroeck JV, Szmania SM, Zhan F, Batchu RB, Spagnoli GC, Tricot G, Pei L, van Rhee F (2002) NY-ESO-1 RNA and protein expression in multiple myeloma is highest in aggressive myeloma and is correlated with chromosomal abnormalities. *Blood* 100:401a
87. Lendvai N, Gnjjatic S, Ritter E, Mangone M, Austin W, Reyner K, Jayabalan D, Niesvizky R, Jagannath S, Bhardwaj N, Chen-Kiang S, Old LJ, Cho HJ (2010) Cellular immune responses against CT7 (MAGE-C1) and humoral responses against other cancer-testis antigens in multiple myeloma patients. *Cancer Immun* 10:4
88. Atanackovic D, Hildebrandt Y, Jadcak A, Cao Y, Luetkens T, Meyer S, Kobold S, Bartels K, Pabst C, Lajmi N, Gordic M, Stahl T, Zander AR, Bokemeyer C, Kroger N (2010) Cancer-testis antigens MAGE-C1/CT7 and MAGE-A3 promote the survival of multiple myeloma cells. *Haematologica* 95:785–793

89. Tinguely M, Jenni B, Knights A, Lopes B, Korol D, Rousson V, Curioni Fontecedro A, Cogliatti SB, Bittermann AG, Schmid U, Dommann-Scherrer C, Maurer R, Renner C, Probst-Hensch NM, Moch H, Knuth A, Zippelius A (2008) MAGE-C1/CT-7 expression in plasma cell myeloma: sub-cellular localization impacts on clinical outcome. *Cancer Sci* 99:720–725
90. Pabst C, Zustin J, Jacobsen F, Luetkens T, Kroger N, Schilling G, Bokemeyer C, Sauter G, Atanackovic D, Marx A (2010) Expression and prognostic relevance of MAGE-C1/CT7 and MAGE-C2/CT10 in osteolytic lesions of patients with multiple myeloma. *Exp Mol Pathol* 89(2):175–181
91. Atanackovic D, Luetkens T, Hildebrandt Y, Arfsten J, Bartels K, Horn C, Stahl T, Cao Y, Zander AR, Bokemeyer C, Kroger N (2009) Longitudinal analysis and prognostic effect of cancer-testis antigen expression in multiple myeloma. *Clin Cancer Res* 15:1343–1352
92. Szmania S, Gnjatich S, Tricot G, Stone K, Zhan F, Moreno A, Thuro B, Melenhorst J, Barrett J, Shaughnessy J, Old LJ, Barlogie B, Brichard VG, van Rhee F (2007) Immunization with a recombinant MAGE-A3 protein after high-dose therapy for myeloma. *J Immunother* 30:847–854
93. Tai YT, Catley LP, Mitsiades CS, Burger R, Podar K, Shringpaure R, Hideshima T, Chauhan D, Hamasaki M, Ishitsuka K, Richardson P, Treon SP, Munshi NC, Anderson KC (2004) Mechanisms by which SGN-40, a humanized anti-CD40 antibody, induces cytotoxicity in human multiple myeloma cells: clinical implications. *Cancer Res* 64:2846–2852
94. Tai YT, Li X, Tong X, Santos D, Otsuki T, Catley L, Tournilhac O, Podar K, Hideshima T, Schlossman R, Richardson P, Munshi NC, Luqman M, Anderson KC (2005) Human anti-CD40 antagonist antibody triggers significant antitumor activity against human multiple myeloma. *Cancer Res* 65:5898–5906
95. Tai YT, Li XF, Catley L, Coffey R, Breitkreutz I, Bae J, Song W, Podar K, Hideshima T, Chauhan D, Schlossman R, Richardson P, Treon SP, Grewal IS, Munshi NC, Anderson KC (2005) Immunomodulatory drug lenalidomide (CC-5013, IMiD3) augments anti-CD40 SGN-40-induced cytotoxicity in human multiple myeloma: clinical implications. *Cancer Res* 65:11712–11720
96. Anderson KC (2003) New agents and approaches in the treatment of multiple myeloma. *Clin Adv Hematol Oncol* 1:151–152
97. Akagi J, Nakagawa K, Egami H, Ogawa M (1998) Induction of HLA-unrestricted and HLA-class-II-restricted cytotoxic T lymphocytes against MUC-1 from patients with colorectal carcinomas using recombinant MUC-1 vaccinia virus. *Cancer Immunol Immunother* 47:21–31
98. Moore A, Medarova Z, Potthast A, Dai G (2004) In vivo targeting of underglycosylated MUC-1 tumor antigen using a multimodal imaging probe. *Cancer Res* 64:1821–1827
99. Treon SP, Mollick JA, Urashima M, Teoh G, Chauhan D, Ogata A, Raje N, Hilgers JH, Nadler L, Belch AR, Pilarski LM, Anderson KC (1999) Muc-1 core protein is expressed on multiple myeloma cells and is induced by dexamethasone. *Blood* 93:1287–1298
100. Lim SH, Chiriva-Internati M, Wang Z, Salati E (2002) Sperm protein 17 (Sp17) as a tumor vaccine for multiple myeloma. *Blood* 100:673a
101. Lim SH, Wang Z, Chiriva-Internati M, Xue Y (2001) Sperm protein 17 is a novel cancer-testis antigen in multiple myeloma. *Blood* 97:1508–1510
102. Ohtomo T, Sugamata Y, Ozaki Y, Ono K, Yoshimura Y, Kawai S, Koishihara Y, Ozaki S, Kosaka M, Hirano T, Tsuchiya M (1999) Molecular cloning and characterization of a surface antigen preferentially overexpressed on multiple myeloma cells. *Biochem Biophys Res Commun* 258:583–591
103. Ono K, Ohtomo T, Yoshida K, Yoshimura Y, Kawai S, Koishihara Y, Ozaki S, Kosaka M, Tsuchiya M (1999) The humanized anti-HM1.24 antibody effectively kills multiple myeloma cells by human effector cell-mediated cytotoxicity. *Mol Immunol* 36:387–395
104. Treon SP, Raje N, Anderson KC (2000) Immunotherapeutic strategies for the treatment of plasma cell malignancies. *Semin Oncol* 27:598–613
105. Noto H, Takahashi T, Makiguchi Y, Hayashi T, Hinoda Y, Imai K (1997) Cytotoxic T lymphocytes derived from bone marrow mononuclear cells of multiple myeloma patients recognize an underglycosylated form of MUC1 mucin. *Int Immunol* 9:791–798

106. Chiriva-Internati M, Wang Z, Salati E, Bumm K, Barlogie B, Lim SH (2002) Sperm protein 17 (Sp17) is a suitable target for immunotherapy of multiple myeloma. *Blood* 100:961–965
107. Lacy HM, Sanderson RD (2001) Sperm protein 17 is expressed on normal and malignant lymphocytes and promotes heparan sulfate-mediated cell–cell adhesion. *Blood* 98:2160–2165
108. Neelapu SS, Kwak LW (2007) Vaccine therapy for B-cell lymphomas: next-generation strategies. *Hematology Am Soc Hematol Educ Program*:243–249.
109. Scallon BJ, Snyder LA, Anderson GM, Chen Q, Yan L, Weiner LM, Nakada MT (2006) A review of antibody therapeutics and antibody-related technologies for oncology. *J Immunother* 29:351–364
110. Stevenson FK, King A, Ottensmeier CH (2003) Vaccine therapy in NHL: future promises and current limitations. *Leuk Lymphoma* 44(Suppl 3):S85–S90
111. Yi Q (2003) Immunotherapy in multiple myeloma: current strategies and future prospects. *Expert Rev Vaccines* 2:391–398
112. Yi Q (2003) Dendritic cell-based immunotherapy in multiple myeloma. *Leuk Lymphoma* 44:2031–2038
113. Barnett BG, Ruter J, Kryczek I, Brumlik MJ, Cheng PJ, Daniel BJ, Coukos G, Zou W, Curiel TJ (2008) Regulatory T cells: a new frontier in cancer immunotherapy. *Adv Exp Med Biol* 622:255–260
114. Ruter J, Barnett BG, Kryczek I, Brumlik MJ, Daniel BJ, Coukos G, Zou W, Curiel TJ (2009) Altering regulatory T cell function in cancer immunotherapy: a novel means to boost the efficacy of cancer vaccines. *Front Biosci* 14:1761–1770

Advances in Biology and Therapy of Multiple Myeloma

Volume 2: Translational and Clinical Research

Munshi, N.C.; Anderson, K.C. (Eds.)

2013, X, 290 p., Hardcover

ISBN: 978-1-4614-5259-1