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Immune Therapies

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Synopsis

Incidents of cancer, particularly in elderly patients living in developing countries, are predicted to raise significantly as the general population rises to an estimated 8.9 billion by the year 2050. Immune cells with specific functions and abilities to localize are vital to cancer prevention. Incidents of multiple myeloma, which is caused by transformed plasma cells, will increase in the coming decades. Combinational treatments prolong the survival of myeloma patients, in turn, escalating the management costs. Even though there have been many accomplishments made in the areas of immune cells and the biology of myeloma, there are still many obstacles in the way of conceptualizing the inter-relationships between immune cells and tumor cells. In order to provide better understanding of these concepts and to move towards improved therapies for myeloma, cell-based therapeutic approaches should be developed. In the following chapter, we will attempt to further illuminate these processes and concepts.

Keywords

Myeloma; immunotherapy; idiotype; dendritic cells

Novel targeted therapies are achieving responses in over 90% of newly diagnosed multiple myeloma (MM) patients; with one third of these patients achieving complete or near complete responses. However, patients still experience disease progression and curative outcomes are rare. This has led to evaluation of novel therapeutic interventions including, immunes-therapy in MM. Allogeneic transplant has provided the rational for development of vaccination strategies. Development of successful immune therapy in MM has been directed at two aspects; first, to develop a successful vaccine that is able to target MM cells with therapeutic efficacy;

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and second, to improve the immune function in MM patients in order to allow robust responses to immune-based intervention[1].

In this chapter, we will provide a brief summary of the status of the immune system in myeloma, and a detailed account of the clinical trials performed with myeloma related antigens including idiotype (Id) alone or in conjugation with other proteins and pulsed with dendritic cells (DCs). Future immunotherapy strategies for the improvement of treatment of multiple myeloma and monoclonal gamapathy of undetermined significance (MGUS) will also be covered.

Obstacles to effective anti-myeloma immunity

With the advancements in immunology, there is better understanding of the interrelationships between MM cells and immune cells. In order to develop effective cancer vaccine, one has to strike a balance between triggering autoimmunity and generating tumor immunity. A low-avidity auto-reactive T cells are present and can be used to mount tumor immunity[2]. In order to generate robust immune responses even against foreign pathogenic antigens, dendritic cells orchestrates an appropriate inflammatory cytokine milieu[3]. In light of the above two requirements, identifying tumor antigens, which are important in eliciting anti-tumor immunity rather than triggering auto-immunity, has been a major obstacle in developing immune-therapy for MM. The approaches pursued are first, to identify patient-specific self antigens that are randomly mutated over time due togenetic instability[4]. These types of antigens do not generally induce auto-immunity and tolerance; however, this approach may be less practical for large-scale applications. The second approach involves use of shared non-mutated self-antigens, which may be prone to develop tolerance when generating anti-tumor immunity. In this context, TERT (telomerase reverse transcriptase) has been targeted to vaccinate cancer patients to reduce tumor evasion[5,6]. Additionally, there is increased realization of the importance of micro-environmental components including stromal cells and cytokines in supporting the tumor cell survival as well as the antigenic determinants that needs to be considered for effective immune-therapy strategy[7]. Finally, in order to optimize immune therapy strategies, multivariable trials evaluating immunological end points along with efficacy should take the place of the traditional clinical trial design for testing cytotoxic drugs[8].

Interactions between immune cells and MM cells

For decades, tumor vaccines have concentrated on generating CTLs (Cytotoxic T Lymphocytes) in order to kill tumor cells[9]. However, growing literature provides convincing evidence that CD4 cells and antibody production increases the efficacy of immune-therapy approaches[10-13]. Further more, one has to take advantage of homeostatic proliferation[14,15] in a lymphopenic host towards developing anti-tumor immunity. Additional role of immune regulation by FOXP3$^+$ (Forkhead box protein 3) CD4 cells[16-23], the influence of cytokine milieu to generate T_H17 cells[24-26] and stress-related NKG2D (Natural-Killer group 2 memberD) ligands (MICA and MICB, MHC-class-I-polypeptide-related sequence A & B)[27,28] in modulating immune responses is being defined. The regulatory T cells that are positive for FOXP3 (natural, expanded & induced) play a critical role in immune homeostasis and are capable of modulating immunity, autoimmunity or tolerance. They predominantly express CD4 and CD25[29] in addition to FOXP3, CTLA-4 (CTL-associated protein 4) and GITR (Glucocorticoid-induced tumor necrosis factor receptor). Their functions are mediated by TGF-β (Transforming growth factor-beta), and vitamin A derivatives[30]; and associated with other transcription factors including NFAT (Nuclear factor of activated T cells), AP1 (Activator protein 1), Runx1 (Runt-related transcription factor 1) and NFkB (Nuclear factor-kappa B)[22,31,32]. Naïve CD4 cells can be differentiated to FOXP3+ cells in the presence of TGF-β and can be differentiated into T_H17 cells in the presence of TGF-β +IL (Interleukin) -6[33,34] and IL-1β or IL-23[24,26]. On the other hand, IL-6 suppresses the induction of FOXP3[34,35]. Complex

networks of cytokines play a crucial role in the differentiation of FOXP3 and/or $T_H17$cells during which antigen-specific immune responses are generated. With reduced $FOXP3^+$ cell number and function, autoimmunity may be observed, while their increased activity may lead to immune-suppression in instead of immunity.

Immuno-biology in myeloma

Multiple myeloma exhibits a number of immune deficiencies in various compartments of the immune system. One has to appreciate the complex nature of the network that prevents the generation of robust immune responses in myeloma patients as represented in Figure 1.

Cell-mediated responses

Myeloma patients have hyperactive T cell responses and deficient antibody responses mediated by B cells[1]. In terms of the expression of various surface markers and functional ability, both CD4 and CD8 T cells seems to be impaired in both MGUS and myeloma [36,37,38], however, the basis for these deficiencies remains unclear. An impairment in the diversity of T cell receptors by defective V-beta repertoire is observed in myeloma following high dose chemotherapy[39]. Even though, specific-cellular and humoral responses against both viral and tumor antigens have been reported in myeloma[40], protective antibody production against pneumonia-causing bacteria is lacking in more than 80% of myeloma patients[41,42]. In addition, myeloma patients showed poor cytotoxic T cell responses against viral antigens in general[40]. A number of studies have shown that idiotype-specific responses can be generated in myeloma patients following vaccination indicating at least partly competent immune system with the ability to generate anti-tumor immune responses is feasible.

Antigen-presenting cells

Antigen-presenting cells (APCs) are generally equipped with antigen processing and presentation as well as co-stimulatory machinery and human leukocyte antigens (HLA) molecules, to generate specific immune responses. We and others have shown in earlier studies that APC are functional in myeloma and can be used for the immune-based clinical studies[43]. In our laboratory, different classes of myeloma proteins pulsed with DCs were utilized in generating *in vivo* anti-myeloma immune responses [44]. However, others have shown impaired DC function for example in one study, DCs failed to up-regulate necessary CD80 expression which may be associated with myeloma progression[37]. This might be due to exposure to cytokines like TGF-β and IL-10. $CD34^+$ DC progenitor cell development is influenced by IL-6 and can be corrected by the treatment with anti-IL-6 antibodies[45]. In addition, in a recent study[46], monocyte-derived DCs from myeloma patients expressed lower levels of important activation surface markers including, CD40, CD80 and HLA-DR and presented recall antigens poorly to T cells. This might be due to a number of cytokines elevated in the tumor microenvironment as well as serum including IL-6, IL-10, TGF-β and vascular endothelial growth factor (VEGF). It is now feasible to improve the defective DC functions in myeloma[47] by *in vitro* DC development techniques and by using innate immune stimulants like CpGs[48,49].

Regulatory T cells

Dysregulation of naturally occurring $CD4^+CD25^+$ T regulatory cells in myeloma at initial diagnosis has been reported[50]. This study indicated lower numbers of $Foxp3^+$ regulatory T cells in PBMC (Peripheral blood mono nuclear cells) of myeloma patients compared to normal healthy volunteers; this was additionally associated with impaired inhibitory responses in MM. Generally speaking, regulatory T cells contribute in establishing homeostasis following specific-immunity and in keeping auto-antigen-based immune responses under control or with a very minimal level of pathological consequences. High IL-6 levels in myeloma may down-

regulate regulatory T cell suppressive activity[34,51]. However, in another study, a higher number of regulatory T cells were reported in myeloma in purified $CD4^{+}CD25^{high}$ population, capable of suppressive activity at 1:2 ratio in a three-way mixed lymphocyte reaction (MLR) following 24 hours of *in vitro* activation[52]. These regulatory T cells were activated away from the tumor microenvironment cytokine milieu prior to evaluation of their functional capacity; and under these conditions, regulatory T cells may have expanded or induced.

T_H17 cells

The role of T_H17 cells in tumor immunity is not well-established[53], however, two cytokines, IL-6 and TGF-β, important in their development, are highly expressed in myeloma. A recent study has reported elevated levels of IL-17 in myeloma compared to normal donor sera. This particular inflammatory cytokine may promote tumor growth via promotion of angiogenesis[54]. Similarly, IL-21, a IL-17 associated cytokine, has been reported to increase myeloma growth and block IL-6 dependent apoptosis in myeloma cell-lines[55].

In spite of the abnormalities and deficiencies observed in myeloma in various compartments of the immune system, anti-tumor immune responses are observed in MM and an ongoing effort is to devise strategies to induce and augment specific responses that may then have clinically relevant effects.

Myeloma clinical trials

As a potential target for immunotherapy, idiotype protein, the immunoglobulin produced in large quantities by myeloma patients, has been extensively studied. These antibody molecules are generated by rearrangement of variable, diversity and joining regions of heavy and light chains. In the past decade, a number of studies have demonstrated a T_H1 type of immunity can be generated using idiotype-pulsed DCs in an HLA-restricted fashion. Thus, these studies demonstrate feasibility of developing idiotype-specific T cell-mediated anti-myeloma responses.

In vivo experimentations

In early 70s, animal experimentations [56] showed that immunizations with purified idiotype proteins were able to produce anti-idiotype antibodies in mice. Further more, when tumor cells that were producing immunized idiotype were transferred to naïve and un-immunized animals, only 11% of animals developed tumors. However, when tumor cells only producing light chains were transferred to naïve animals such protection was not observed.

Pre-clinical studies

Yi et al[57] reported that T cells from myeloma patients were capable of responding to autologous idiotype *in vitro*. Specific-idiotype mediated IFN-γ (Interferon-gamma) and IL-2 production was observed in 66% and 76% of T cells respectively, from myeloma patients. Idiotype-specific proliferation was observed in 36% of the patients tested. These results indicated that the T cells against self-idiotype can be used to generate T cell responses and provided the rational to use the idiotype as a tumor-specific target for vaccination studies. In addition, an another study by King et al[58] showed that mice immunized with DNA vaccine consisting of idiotype and fragment C of tetanus toxin, 70% of animals survived for more than two months after challenge with tumor cells compared to vehicle control animals. This particular study indicated that DNA fusion vaccines could be effective in generating protective immunity against tumors. Stritzke et al[59] have reported that when animals were vaccinated with tumor cells along with GM-CSF, followed by IL-2 administration, the tumor recurrence was delayed and survival improved compared to control animals. This indicated that NK cells and CD8 T cells were important in generating tumor-specific immunity to render the protection in these animals tested.

Idiotype-based clinical results

In order to vaccinate patients with a given antigen to generate immune responses, one has to have a large quantity of clinical grade antigen and that is specific to the tumor. It is easy to purify large amount of monoclonal para-protein or idiotype from the serum of myeloma patients. As shown in Table 1, a number of clinical trials using idiotype alone or in combination with cytokines as tumor antigen to vaccinate myeloma patients have been performed. Bergenbrant et al[60] used myeloma para protein alone to repeatedly immunize five myeloma patients. T cells following vaccination produced higher levels of IFN-γ in response to idiotype protein. However, with repeated immunizations, T cell responses appeared to be rare and patients did not achieve clinical responses following vaccination. Osterborg et al[61] immunized five myeloma patients with autologous idiotype protein along with GM-CSF and although *in vitro* studies showed that both CD4 and CD8 cells produced IFN-γ and IL-2 following vaccination, significant T cell proliferative and DTH responses were not observed. Changes in para-protein levels were also not reported in these immunized patients. Rasmussen et al[62] have administered seven vaccinations to six myeloma patients with idiotype and IL12 with or without GM-CSF (Granulocyte-macrophage colony-stimulating factor). Immediately following vaccination, clonal B cells went down and most of the patients showed specific-T cell responses. However, after 30 weeks post-vaccination, T cell responses were diminished and para protein levels were elevated. In another study by Bertinetti et al[63], three auto SCT myeloma patients were given four immunizations of idiotype with GM-CSF in addition to hepatitis B vaccine. Even though partial clinical remission was observed in vaccinated patients, it did not correlate with T cells responses and hepatitis B antibodies were not detected following vaccination. In a long-term study, Massaia et al[64] and Coscia et al[65] vaccinated 12 myeloma patients with idiotype with KLH (Keyhole limpet hemocyanin) and GM-CSF following high dose chemotherapy and autoSCT (Auto-stem cell transplant) in first CR (Complete response). Even though, a majority of the patients (85%) showed DTH (Delayed-type hypersensitivity) response, T cell responses and anti-idiotype antibodies were not significantly increased following vaccinations and no clinical benefit was observed following vaccinations. Using allogeneic transplantation settings, we have vaccinated[66] HLA-matched sibling donors first with Idiotype and KLH in addition to GMCSF prior to bone-marrow collection and transplant. The rationale for vaccinating BMT (Bone-marrow transplant) donors prior to transplantation is to transfer tumor-specific immune components with the graft. After BMT, patients received three booster vaccinations. Three out of five patients after vaccination showed idiotype-specific T cell responses, improved clinical response from partial remission prior to BMT and Idiotype vaccinations to CR and maintenance of response up to 5-8 years. In autologous transplant setting, we have conducted a large study with three different cohorts and a total of fifty myeloma patients have been enrolled. In this study, patients following double auto transplant received three or six vaccinations with idiotype coupled with KLH with GM-CSF in first two cohorts. A third cohort was vaccinated before and after transplantation. The majority of the patients (58%) following vaccinations produced idiotype-specific T_H1 type of responses and had an elevated proliferative response in addition to DTH responses (42%).

In summary, these studies demonstrated that myeloma patients do respond to vaccination and that idiotype is a weak immunogen, however, the responses are not robust, do not persist for prolonged periods of time and clinically meaningful outcomes have not been observed. This has prompted investigation of novel approaches and antigens for immunotherapy.

Idiotype-pulsed DC trials in myeloma

The majority of clinical trials conducted using idiotype-pulsed DCs showed immune response, yet significant clinical responses are lacking (Table 2). Lim et al[67] showed that following three idiotype-pulsed DC vaccinations, both idiotype-specific-T cell and anti-idiotypic-antibody responses were observed, however, none of the vaccinated patients showed clinical

improvements. Titzer et al[68] vaccinated eleven myeloma patients with idiotype-pulsed with CD34-derived DCs. T cells from only four out of ten patients were able to show antigen-specific production of cytokines following vaccination and clinical responses were not observed following vaccination.

Liso et al[69] immunized twenty-six patients following high dose chemotherapy with idiotype-pulsed DCs with or without KLH. Only four out of twety-six patients showed specific-proliferative responses, however, seventeen patients are alive at 30 months post-vaccination. Yi et al[70] vaccinated five patients at remission following high dose chemotherapy. Three idiotype-pulsed monocyte-derived DC vaccinations were administered followed by IL-2 therapy for five days. Four out of five patients showed Idiotype specific-T cell cytokines production and one patient achieved partial response (PR) following vaccination. Reichardt et al[71] vaccinated twelve patients post autoSCT with idiotype-pulsed DCs. In terms of T cell responses, only two out of twelve demonstrated idiotype-specific proliferation and one of three patients showed specific CTL-mediated killing. No clinical improvement was reported following vaccinations[72]. Recently, Bendandi et al[73] conducted a clinical trial using DCs loaded with idiotype in four myeloma patients following reduced intensity conditioning alloSCT. Anti-idiotype antibody responses were not seen. T cell responses by production of T_H1 cytokines were noted in two out of 4 patients. Three patients in this study had transient responses and one patient stable disease. Recently, sixteen myeloma patients have been vaccinated following autoSCT with irradiated autologous myeloma plasma cells and genetically modified K562 cells to produce GM-CSF. Of the sixteen patients that have completed the study, six showed CR and five with PR after autoSCT and vaccination without noticeable toxicities. Both cellular and antibody responses have been observed in addition to DTH responses.

In summary, generating anti-tumor immunity is feasible, yet convincing clinical efficacy is lacking in myeloma even with DC-pulsed idiotypic vaccinations. Since intravenous DC vaccinations lead to its sequestration[74,75], one can improve DC migration patterns by subcutaneous administration to generate protective T_H1 type responses [76]. Since immature DCs are unstable when necessary cytokines are withdrawn[77], the use of mature DC derived from peripheral blood monocytes would be superior in presenting antigens to T cells. Even though idiotype is a weak immunogen, one can generate adequate immunity against this type of tumor-specific antigen both post-autologous transplantation as well as donor vaccinated post-allogeneic stem cell transplantation.

Future directions

Targeting a single tumor-specific antigen will allow tumor cells to become resistant by mutation of a particular gene and by evading immunity. In order to avoid this obstacle, one has to use approaches directed at multiple antigenic targets. One example is pulsing DCs with whole cell-myeloma lysates or fusing DCs with myeloma cells. A pre-clinical study using animal cells and human cells have confirmed the feasibility of presenting a wide array of myeloma-related antigens by fusing myeloma cells with DCs for the development of effective CTLs[78]. A clinical study of MM/DC fusion cell vaccination with GM-CSF is ongoing. Eleven patients have been enrolled in this study and demonstrated that adequate number of functional DCs can be obtained to vaccinate patients three or more times without toxicity. The majority of the patients were stabilized with tumor-specific T cell responses by increased production of IFN-γ following vaccination.

As production of patient specific vaccination is difficult, there is an ongoing effort to identify a cocktail of antigens, which can be used in universal fashion for all the patients. A number of investigators have identified novel myeloma-specific antigens by screening myeloma cDNA

expression libraries using the SEREX (Serological expression cloning) technique for eventual development of an antigen cocktail. With this technology, myeloma–specific antigens such as XBP1, OFD1 (Orofaciodigital 1), BCMA (B-cell maturation receptor) and ROCK1 (Rho-associated kinase 1) are identified. Recently, immune responses directed at an embryonic stem cell marker, SOX2 expressed in the CD138- compartment in MGUS patients have been reported. Such responses may help in predicting clinical out-comes based on the SOX2–induced immunity[79]. Furthermore, other trans-membrane proteins, including MUC1, previously reported to be expressed in glandular epithelial cells[80], have been shown to be over-expressed in myeloma cell-lines and primary myeloma cells[81,82]. Malignancy-associated cancer-testis antigens including, MAGE family: BAGE, GAGE, PRAME, NY-ESO-1, and Sperm protein17[83], generally, not expressed in normal tissues, have been shown to be expressed in myeloma. These have been targeted for active investigation for cancer immunotherapy because of their tumor-specific expression and the ability to induce tumor-specific immunity. However, the vaccinations with these cellular antigens generating significant clinical response have yet to be seen. In addition, since most of these cellular antigens are present in the late phase of myeloma and associated with only a subset of myeloma cells, using them for vaccination may not translate into clinical efficacy.

The ongoing future approach to obtain clinically effective vaccination includes methods to increase the immunogenicity of DCs, to apply methods to extend tumor immunity following vaccinations, to develop approaches to improve immune status in myeloma patient, to utilize homeostatic proliferation to generate efficient tumor immunity, to establish regulatory T cells homeostatic functions to the normal thresh-hold level, and to abrogate T_H17-mediated immunopathology. Eventually, a combination of vaccination with tumor-specific antigens, adoptive transfer of T cells cultured with immunomodulatory agents will provide the robust and sustained immune responses with clinical efficacy.

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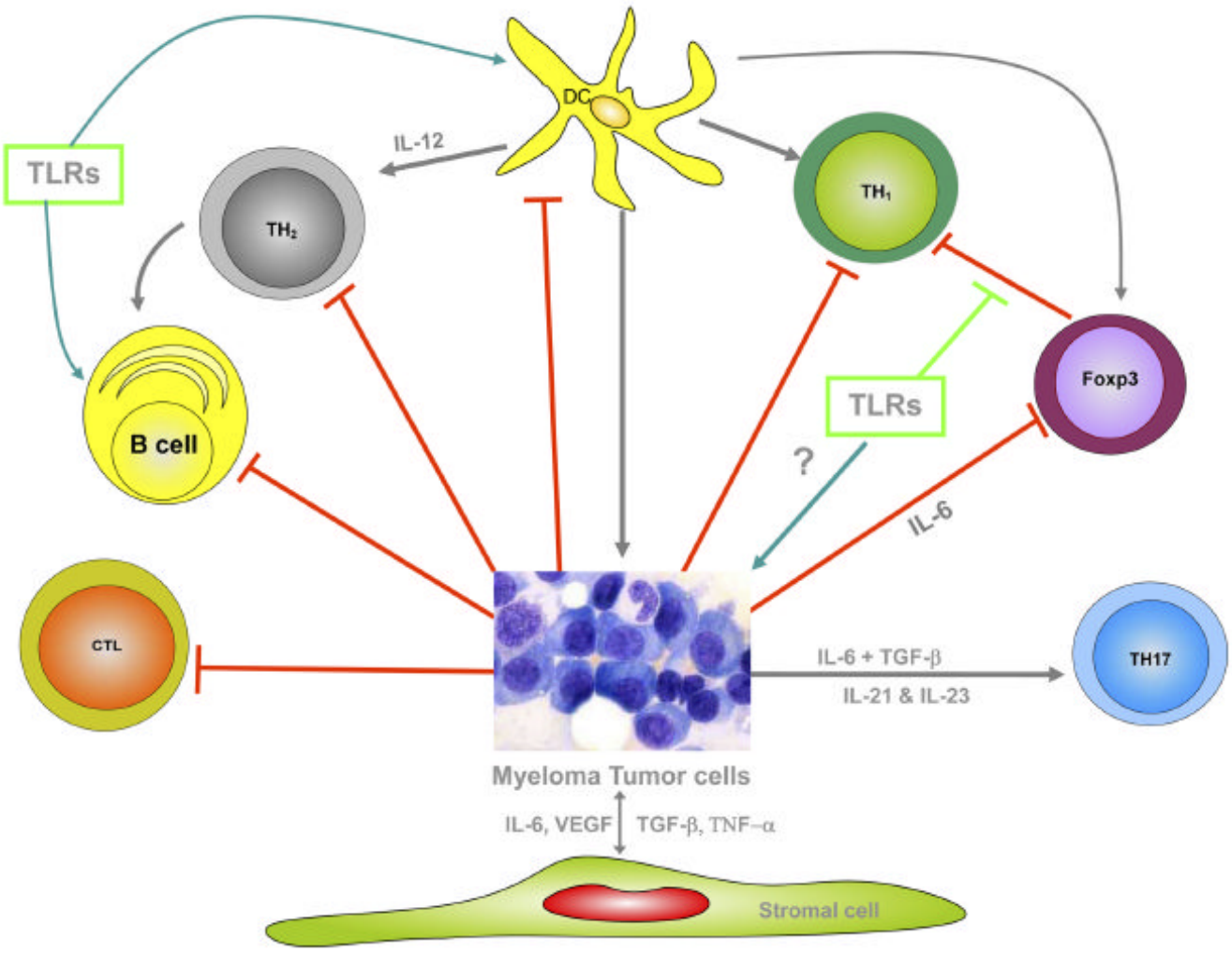

Figure 1.

Table 1

Idiotype-based clinical trails in Myeloma

Vaccine	Patients	Cellular Responses		Clinical response	Author (Ref #)
		T cell	B cell		
Id alone Repeated Vaccines	5	3/5	+	No Response	Bergenbrant et al. ([60])
Id + GM-CSF 6 Vaccines	5	1/5	-	Para protein levels unchanged	Osterborg et al. ([61])
Id +KLH + GM-CSF (+IL-2) AutoSCT at Remission	12 T cell HD Chemo	2/11	-	Para protein levels unchanged	Massaia et al., Coscia et al. ([64,65])
Id + IL-12 +/- GM-CSF 7 Vaccines	6	5/6	-	No change in Para protein	Rasmussen et al. ([62])
Id +GM-CSF 4 Vaccines + HepB Vac	3 autoSCT	1/3	-	No abs to HepB	Bertinetti et al. ([63])
Tumor cell + GM-CSF-K562 8 Vaccines after AutoSCT	16	3/5	+	3/16 rise in Para Protein	Borrello et al. (ASH presented)
Id + KLH +GM-CSF, 6 vaccines	5, AlloSCT	4/5	+	3/5 Stable CR	Neelapu et al. ([66])
Id + KLH + GM-CSF, 3 or 6 vaccines	50 autoSCT	28/48	+	No response	Munshi et al. (ASH presented)

Abbreviations: Id, Idiotype; KLH, keyhole limpet haemocyanin; AutoSCT, Autologous Stem Cell Transplantation; GM-CSF, Granulocyte-Macrophage Colony Stimulating Factor; IL-12, Interleukin-12; HD, High Dose; HepB, Hepatitis B vaccine; CR, Complete Response.

Table 2

Clinical trails using DCs pulsed with idiotype of Myeloma patients

Vaccines	Patients	Clinical Outcome	Authors (Ref#)
7 Vaccines (Id + KLH +DCs) (2-iv Id + DCs & 5- Id + KLH)	12 AutoSCT High Dose Chemotherapy	Stable	Reichardt et al. ([71],[72])
3 Vaccines (Id +DCs)	6	Progressed	Lim et al. ([67])
7 Vaccines (2-Id + DCs & 5-Id+ KLH)	26, High dose chemotherapy, AutoSCT	17 Live/ Stable	Liso et al. ([69])
4 Vaccines (1-Id + DCs & 3-Id +GMCSF)	11	Progressed	Titzer et al. ([68])
3 Vaccines (Id +DCs + IL-2)	5 High dose Chemotherapy at Stable PR	4 Stable/1 Relapsed	Yi et al. ([70])
4-7 Vaccines (Id + KLH + GMCSF)	4 AlloSCT	3 Progressed	Bendandi et al. ([73])

Abbreviations: Id, Idiotype; KLH, keyhole limpet haemocyanin; DCs, Dendritic cells; AutoSCT, Autologous Stem Cell Transplantation; GMCSF, Granulocyte-Macrophage Colony Stimulating Factor; PR, Partial response, & IL2, Interleukin 2