Minireview

Current Clinical and Laboratory Strategies to Augment the Efficacy of Immunotherapy in Multiple Myeloma[1]

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Abstract

Multiple myeloma is still an incurable, lethal disease for the vast majority of patients. Myeloablative chemotherapy combined with autologous or allogeneic hematopoietic stem cell transplantation only partially met the great expectations initially set in its efficacy and is associated with a high level of toxicity. However, the considerable progress in understanding the biology of multiple myeloma led to the development of promising molecular therapies. Numerous immunotherapy-based approaches are currently evaluated in clinical trials. Moreover, remarkable progress has been achieved in gene therapy during the last decade, and the repertoire of gene transfer techniques can be expected to improve continuously. Gene transfer is increasingly applied in biological therapies in multiple myeloma. This article reviews the currently applied clinical and laboratory strategies to augment the efficacy of immunotherapy in multiple myeloma and aims to define its perspectives in multimodality treatment of multiple myeloma.

Introduction

MM[3] (1) caused by the proliferation of malignant plasma cells is still a lethal disease for most of the patients. Despite considerable progress in understanding its biology and introduction of high-dosage chemotherapy, the disease has remained incurable except for a very small proportion of younger patients who can be treated with allogeneic transplantation. Despite considerable efforts toward improving the safety of transplant procedures, the treatment-related mortality of ~40% is very high (1). Standard dosage chemotherapy regimens result in median survival rates of 2–3 years. High-dose myeloablative chemotherapy followed by autologous PBSCT has significantly improved survival of patients younger than 65 years as compared with conventional chemotherapy. Unfortunately, molecular remissions are rare (2), and virtually all patients seem to relapse (3). Survival curves do not yet reach a plateau, and, therefore, this toxic therapy seems to be not curative.

Because chemotherapy is not expected to offer a cure or long-term disease control without considerable toxicity, the development of new therapeutic strategies is required. MM appears to be particularly suitable for immunotherapy because myeloma cells express tumor-associated and even tumor-specific antigens and both allogeneic and autologous immune responses have been detected (4). The chance for cure by allo-SCT is probably mediated by the immunological GVM effect. Van Baren *et al.* (5) detected expression of genes of the *MAGE, BAGE, GAGE* and *LAGE-1/NY-ESO-1* families that could serve as targets in specific immunotherapy approaches in a high proportion of bone marrow samples from patients with advanced MM. However, myeloma cells may escape from immune surveillance by down-regulation of TAAs, MHC antigens and costimulatory molecules, or by secretion of immunoinhibitory factors such as TGF-β (6, 7). Immunotherapeutic approaches aim at augmentation of presentation of TAAs to CTLs, stimulation and proliferation of cytotoxic cells by cytokines, or generation of antibody-dependent cytotoxicity. The interest of most investigators concentrated on cytokine therapy, immunomodulatory agents, monoclonal antibodies, vaccination strategies, adoptive immunotherapy, and gene therapy.

Gene therapy has the potential to expand the armament of immunotherapy in MM considerably. Moreover, gene therapy offers several nonimmunological strategies such as transduction of wild-type tumor suppressor genes and suicide genes or blocking of oncogenes by antisense therapy. This review provides an overview of recent preclinical and clinical studies applying molecular therapy in MM and focuses on the synergisms of immunotherapy and gene therapy.

Immunotherapy in MM

IFN-α, Thalidomide, and Monoclonal Antibodies. IFN-α and other IFNs have a direct and dose-dependent inhibitory effect on the proliferation of myeloma cells *in vitro*. They have been shown to down-regulate expression of c-*myc* and N-*ras* oncogenes. Immunomodulatory effects such as activation of NK cells may also be implicated. Randomized clinical trials have produced differing results. A recent meta-analysis based on individual patient data showed a significant improvement of a

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[3] The abbreviations used are: MM, multiple myeloma; SCT, stem-cell transplantation; allo-SCT, allogeneic SCT; GVM, graft-*versus*-myeloma; PBSCT, pheripheral blood SCT; IL, interleukin; TAA, tumor-associated antigen; NK, natural killer; PR, partial response; CR, complete response; DLI, donor lymphocyte infusion; GVHD, graft-*versus*-host disease; GM-CSF, granulocyte macrophage colony-stimulating factor; PBMC, peripheral blood mononuclear cell; CIK cell, cytokine-induced killer cell; SCID, severe combined immunodeficiency; TGF-β, transforming growth factor β; DC, dendritic cell; Id, idiotype; KLH, keyhole limpet hemocyanin; MRD, minimal residual disease; HSV-TK, herpes simplex virus thymidine kinase; HLA, human leukocyte antigen; scFv, single-chain variable fragments; TCR, T-cell receptor; APC, antigen-presenting cell.

3-year survival of 6% by maintenance therapy with IFN-α (8). However, this slight benefit of IFN-α has to be weighed against marked side effects, such as fever, fatigue, flu-like syndrome, or thrombocytopenia, and considerable costs.

Thalidomide, which has antiangiogenic properties, may become an important agent in the treatment of MM. Several other mechanisms of action such as induction of CD8 Th1 T-cell response, alteration of the secretion of cytokines, direct influence on the growth of myeloma cells, and modulation of adhesion to the bone marrow stroma have been proposed (9). Recent studies showed response rates of ~25–30% in patients with refractory disease (10). The toxicity is very moderate. Numerous clinical trials are ongoing to determine the role of thalidomide in various treatment situations.

Monoclonal antibodies are a therapeutic option of increasing importance for patients with lymphoma and several other malignancies. Particularly, the humanized anti-CD20 antibody rituximab showed excellent results in B-cell lymphomas. In a large Phase II multicenter trial, 48% of the patients with relapsed low-grade lymphoma responded (11). Compared with chemotherapy, the toxicity was mild. The combination of this antibody with chemotherapy is advantageous. For example, in the Phase III GELA-trial, rituximab increased the CR rate of cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP) chemotherapy in elderly patients with diffuse large B cell lymphoma from 60 to 76% and the 12-month overall survival from 68 to 83% (12). Although CD20 is present only on ~20% of myeloma cells (13), it is detectable in clonogenic B cells, which circulate in most patients and which are suspected to be myeloma precursor cells because they bear identical immunoglobulin heavy chain rearrangements. However, Treon *et al.* (14) reported only one PR and five stable disease among 19 evaluable myeloma patients treated with rituximab. Interestingly, up-regulation of CD20 expression in myeloma cells by IFN-γ had been observed, suggesting the clinical evaluation of IFN-γ pretreatment before rituximab administration (15). Cremer *et al.* (16) presented preliminary results of a Phase I/II study of rituximab as consolidation therapy after PBSCT. In three patients with progressive disease after PBSCT, no response was induced by rituximab. Ten patients remained in CR or PR. Two patients with CR relapsed. The median follow-up after PBSCT was 28 months (16). Taken together, rituximab is probably less effective in MM compared with B-cell lymphoma but patient numbers are still much too low at the present time to draw final conclusions.

Several other surface antigens on myeloma cells, including CD19, CD20, CD38, CD40, CD45, CD54 (ICAM-1), CD138 (human syndecan 1), HM1.24, MUC1 core protein, sperm protein 17, and the immunoglobulin Id, that may be suitable for antibody-directed treatment have been proposed (9, 15, 17). However, clinical trials of antibody treatment are in very early stages. A Phase II study using an anti-CD19 antibody coupled with the immunotoxin ricin showed no response in five patients treated (18). Hamblin *et al.* reported on the first clinical experience with anti-CD38 antibody in four patients. However, to date, minimal clinical activity has been observed (19).

Cellular Therapy. The transfer of cytotoxic effector cells termed "adoptive immunotherapy," has been shown to be a promising new strategy for treatment of various hematological malignancies, including leukemia and non-Hodgkin's lymphoma (20). Two alternative approaches, DLIs after allo-SCT and adoptive cell therapy using autologous T cells have to be distinguished.

The high treatment-related mortality after allo-SCT is a persistent problem. However, the relapse rate is lower than with autologous PBSCT, molecular remissions are frequent (3) and the survival rates of patients surviving the first post-transplant-year are higher compared with autologous PBSCT (21). Recent studies demonstrated the efficacy of DLIs in patients suffering from relapses of MM after allo-SCT (22). Taken together, these observations demonstrate a GVM effect that is related to the well-known graft-*versus*-leukemia effect (23, 24). Lokhorst *et al.* (25) administered DLIs in 27 patients with relapsed MM to enhance the GVM effect. Fourteen patients (52%) responded, 6 (22%) of whom achieved a CR. Five patients responded to T-cell dose escalation. Major toxicity was acute and chronic GVHD.

Approaches to further enhance the efficacy of DLIs are currently investigated in several malignancies. In a mouse leukemia model, Luznik *et al.* (26) showed that DLI followed by a tumor vaccine (irradiated C1498 acute myelogenous leukemia cells mixed with a GM-CSF-producing bystander cell line) resulted in significantly prolonged tumor-free survival compared with DLI alone. Moreover, *in vitro* immunization of donor PBMCs using Id-pulsed DCs to generate tumor-specific donor T cells has been proposed (27). Li *et al.* (28) immunized two healthy human sibling stem cell donors with s.c. administered KLH-conjugated Id proteins from their recipients. *In vitro*, T cells from the immunized donors released high levels of T helper 1-type cytokines in response to stimulation with myeloma cells from their recipients, which suggested specific donor immunization. Additional studies aimed at increasing efficacy and reducing toxicity of allo-SCT and DLI by enhancing antigen specificity of donor T cells are necessary. The mechanisms involved in the GVM effect have not been clearly determined yet. Orsini *et al.* (22) demonstrated the appearance of clonal T-cell populations in each of the three patients responding to DLI, by molecular analysis of the TCR repertoire using PCR. Further functional characterization of these distinct clonal expansions, especially examination as to whether alloreactive T cells are directed against minor histocompatibility antigens or TAAs, is required.

Considerable progress has been made in the last years in the *ex vivo* generation of large numbers of cytotoxic cells for autologous adoptive immunotherapy. We developed a protocol that enables efficient *ex vivo* expansion of "cytokine-induced killer" cells. CIK cells are cultured from peripheral blood lymphocytes in the presence of IFN-γ, IL-2, monoclonal antibody against CD3, and IL-1β. CIK cells have been found to be highly effective in a SCID mouse lymphoma model (29) and in purging autologous bone marrow in patients with chronic myelogenous leukemia (20). Furthermore, we conducted a Phase I clinical study applying autologous CIK cells transfected with the *IL-2* gene in patients with metastatic renal cancer, colorectal cancer, and lymphoma. The treatment was well tolerated, and transfected cells could be detected for up to 2 weeks after infusion. There was a significant increase in serum levels of IFN-γ, GM-CSF, and TGF-β during treatment. Concerning clinical

outcome, six patients remained in progressive disease, three patients developed stable disease by treatment but one patient with lymphoma developed a CR (30). Recently, Leemhuis *et al.* (31) presented early data from a Phase I study in which nine patients with advanced Hodgkin's disease or non-Hodgkin's lymphoma, all of whom failed autologous transplantation, were treated with escalating doses of CIK cells. Toxicity was minimal. Two patients achieved PR, and two patients had stabilization of disease. Apart from these results, CIK cells are a promising approach for adoptive cell therapy in MM because tumor cell lines expressing P-glycoprotein, which is a major cause of chemotherapy failure, have been shown to be sensitive to CIK cells (20).

Lokhorst and Liebowitz (32) reported on an alternative approach currently being tested in patients with chemotherapy-resistant lymphoma and planned in patients with MM. Autologous T cells were obtained before autologous SCT, were expanded using an anti-CD3/CD28 culture system, and were reinfused 14 days after transplantation. All six high-risk patients responded to combined therapy. However, two patients developed severe autoimmune toxicity, which necessitated immunosuppressive therapy. This indicates that various problems have to be solved in adoptive therapy. Particularly, the development of techniques that allow large-scale generation of tumor-specific CTLs to increase efficacy and to minimize toxicity is needed.

Vaccination Strategies. Numerous strategies of myeloma vaccination are under investigation. These include idiotypic paraprotein, DCs pulsed with paraprotein, whole myeloma cell vaccines, and myeloma cell-DC fusions. Immunoglobulins contain unique regions commonly termed Ids that can be recognized by the immune system. The Id-determinant in the paraprotein isolated from the serum is a tumor-specific antigen and a promising target for immunotherapy. Id-specific T cells have been demonstrated in most MM patients (4). They recognize processed Id-determinants presented by MHC class II molecules on APCs (33).

In follicular lymphoma, long-term vaccine-induced molecular remissions followed by Id vaccination have been documented (34, 35). In MM, clinical studies are less far advanced. Yi and Osterborg (33) immunized MM patients with the autologous M-component precipitated in aluminum. Three of five patients showed an induction of specific cellular and humoral immunity. Osterborg *et al.* (36) immunized five patients with serum M-component by repeated intradermal injections together with GM-CSF. All of the patients developed an Id-specific T-cell immunity, defined as blood T cells predominantly secreting IFN- γ and IL-2. Massaia *et al.* (37) reported on a vaccination trial using Id-proteins conjugated to KLH and low doses of s.c. GM-CSF or IL-2 as immunoadjuvants in 12 patients in remission after high-dose chemotherapy and PBSCT. Id-specific T-cell proliferative responses were documented in 2 patients, whereas an Id-specific delayed-type hypersensitivity reaction was observed in 8 of 10 patients studied. Schuetze *et al.* (38) conducted a similar trial in patients responsive to autologous or allogeneic transplantation who received a s.c. Id-KLH vaccine combined with GM-CSF. Six of eight patients vaccinated more than 100 days posttransplant developed a cellular immune response to Id, whereas four patients vaccinated before 100 days posttransplant did not. These data indicate that the immune competence status of MM patients may be still susceptible to specific immunization after high-dose chemotherapy and PBSCT. However, median observation times were relatively short, and it remains to be determined whether generation of Id-specific immune responses can reduce the relapse rate of patients with MRD.

DCs are regarded as the professional APCs capable of initiating T-cell and B-cell responses, especially priming naive CTLs. Therefore, DCs may play a key role in the development of immunotherapeutic strategies for MM. However, many tumors are able to avoid DC-mediated immune surveillance, probably by inhibition of the maturation and activation of DCs (39). Isolation of DCs from peripheral blood or bone marrow, subsequent culture with TAAs (pulsing) and growth factors (IL-4, GM-CSF, tumor necrosis factor α), and readministration to the patient is expected to enhance DC-mediated antitumor CTL activation. In addition to Ids, whole myeloma cells or cell lysates can serve as alternative pulsing agents. They may have the additional advantage of containing a variety of as-yet-unidentified TAAs. Numerous groups examined DC-vaccines *in vitro* and in mouse models. For example, Osman *et al.* (40) showed that blood-derived DCs, pulsed with myeloma cell extract, activated specific CTLs and induced them to kill autologous myeloma cells *in vitro*. Most clinical studies demonstrated PBMCs or T-cell-proliferative responses to Id, and some observed development of anti-Id antibodies (Table 1). Various vaccine preparations were used. Side effects were mild or absent. However, to date, clinical responses have been limited and additional observation time is required.

Alternative approaches to Id-vaccination include DC-pulsing with whole myeloma cells or MUC1 protein and myeloma-cell/DC fusions. Lappin *et al.* (41) demonstrated *in vitro* efficacy of DCs pulsed with a myeloma cell line (U266) in which apoptosis was induced by UVB irradiation. Brossart *et al.* (42) were able to induce CTL lysis of various carcinoma cells in an antigen-specific fashion after DC-pulsing with MUC1 protein. Because MUC1 protein is expressed in myeloma cells, this approach should also be evaluated in MM. Raje *et al.* (43) developed a new approach of fusing whole myeloma cells and DCs. The resulting hybridoma cells are expected to present both TAAs in a MHC class I fashion and DC-derived costimulatory molecules. The efficacy of this approach had been shown previously in a breast cancer mouse model (44). First results from six patients show that hybridoma cells, but not myeloma cells or DCs alone, were potent stimulators of autologous patient T cells. Autologous PBMCs triggered by hybridoma cells demonstrated MHC-restricted, myeloma-specific cytolysis (43). These experiments provide the basis for clinical studies using these hybridoma cells for vaccination in MM. Finally, the telomerase catalytic subunit, which can be recognized by CTLs (45), is an interesting target for myeloma immunotherapy. This is even more important because telomerase activity is elevated in myeloma cells and may play a role in the malignant transformation (46).

In the future, intensified basic research is needed to better understand host-tumor interactions. For instance, Brown *et al.* (7) found that there is a normal number of DCs in the blood of patients with MM, but in patients with progressive disease, DCs fail to up-regulate CD80 expression because of TGF-β1 and/or

Table 1 Clinical studies on Id paraprotein-pulsed DC vaccines in MM

First author (Ref.)	No. of pts[a]	Setting	Immune responses	Clinical responses	Toxicity
Lacy (92)	18	After PBSCT: residual disease, 17 pts; CR, 1 pt	Id-specific T-cell proliferation was detectable after the fourth vaccine in all responding pts.	CR, 3/17 pts; PR, 2/17 pts	None
Wen (93)	1	Advanced-stage refractory myeloma	Id-specific T-cell proliferative responses with production of cytokines and anti-Id antibodies.	Transient minor fall in the serum paraprotein level	None
Lim (94)	6	IgG myeloma	PBMC proliferative responses to Id (5 pts), IFN-γ production (2 pts). Increases in CTL-precursor freq. for Id-pulsed autologous targets (3 pts); anti-Id IgM (3/5 pts), anti-Id IgG (4/5 pts).	Modest (25%) but consistent drop in the serum Id level (1 pt)	None
Reichhardt (95, 96)	12	After PBSCT: PR, 11 pts; CR, 1 pt	KLH-specific cellular proliferative responses (all pts) Id-specific, cellular proliferative immune response (2/9 pts), Id-specific CTL-response (1 pt).	Declining Id levels (3/9 pts)	Local and transient reaction after s.c. injection
Titzer (97)	11	Advanced-stage myeloma	Increased anti-Id antibodies (3/10 pts), Id-specific T-cell responses (ELISPOT) (4/10 pts).	Decreased plasma-cell infiltration in the bone marrow (1 pt)	None
MacKenzie (98)	42	Progression after standard therapy, 28 pts; progressive disease after PBSCT, 14 pts	Not reported (abstract).	CR or PR was not reported. Minor decreases in paraprotein (6 pts). Disease stabilization >35 weeks (10 pts)	In 11.9% of pts (headache, 2; sore arm, 1; dyspnea, 2)

[a] pt, patient; freq., frequency.

IL-10. The development of vaccine strategies should include appropriate assays to monitor treatment results, optimal vaccine formulation, and timing of vaccine administration to increase the proportion of patients achieving Id-specific immune responses. The clinical benefits of Id vaccination in MM remain to be determined in controlled studies with longer observation periods.

Gene Therapy in MM

Gene Therapy in Cancer. Remarkable progress has been made in the field of gene therapy during the past decade, and the techniques of gene transfer can be expected to improve continuously. Despite the fact that gene therapy to date is not established in cancer treatment, its enormous potential generates the increasing interest of clinicians. However, many technical obstacles need to be overcome. Particularly, transfection efficiencies and targeting to malignant cells have to be improved (47, 48). Furthermore, it is still not clear whether it is safe to incorporate genes into nuclear DNA and whether, therefore, an oncogenic risk is associated with retroviral gene transfer (49).

Strategies of gene therapy in cancer can be divided into immunological and nonimmunological methods. The latter include the inserting of wild-type tumor suppressor genes such as *p53*, transduction of suicide genes such as *HSV-TK*, blocking the activity of oncogenes by antisense therapy, and protecting the normal host tissue from toxic effects of chemotherapy by transfection of drug-resistance genes. Furthermore, gene marking of malignant and normal hematopoietic cells to monitor the efficacy of conventional therapies can be integrated here.

There are three immunological approaches: (*a*) gene transfer in tumor cells could enhance immunogenicity; (*b*) APC transfection could improve tumor antigen presentation; or (c) transfection of immunological effector cells could increase cytotoxicity against myeloma cells. Most of the gene therapy strategies currently investigated in MM are based on immunotherapy because this malignancy is proven to be immunogenic (4, 5) and is expected to circumvent immune escape mechanisms by gene transfer.

Gene Transfer in the Therapy of MM: Preclinical Studies. Effective transfection of the applied cells is generally a prerequisite for a successful gene therapy strategy. Various viral gene transfer methods have been used to transfect myeloma cells. A study comparing the efficacy of different transfection methods in myeloma cells is still lacking. Björkstrand *et al.* (50) have shown first that retroviral-mediated gene transfer into human myeloma cells is feasible. However, only low transduction efficiencies of 1.5–3.8% could be achieved in this study, probably caused by a low proliferation rate of myeloma cells. Furthermore, a lentivirus (51) and an adeno-associated virus (52) have been used. The highest transfection efficiencies of up to 98% have been reported using adenoviral vectors (53, 54) ,which may be the preferable vectors to transfect myeloma cells because of their ability to transduce nondividing cells (55). However, it has to be considered that adenoviral proteins may act as targets for the immune system and, thus, may cause elimination of the transfected cells by the host's immune response in repeated vaccinations (56).

Nonviral vectors are poorly investigated in MM. Early

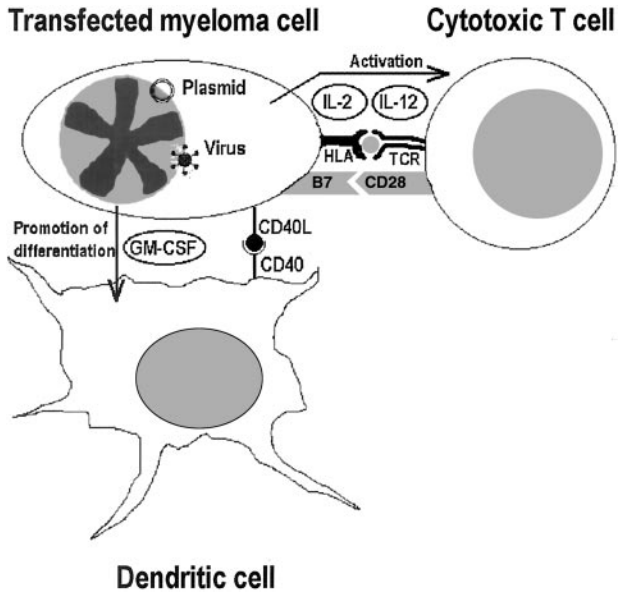

Fig. 1 *In vivo* effects of plasma cell vaccination. Interactions of DCs and cytotoxic T cells with myeloma cells after transfection with various transgenes encoding GM-CSF, IL-2, IL-12, B7–1/-2, and/or CD40 ligand (*CD40L*).

studies demonstrated reporter-gene expression after recombinant plasmid vector transduction using fusion of bacterial protoplasts with myeloma cell lines (57). However, transformation frequencies of 10^{-3} to 10^{-4} were very low, and this approach is not applicable to gene therapy for safety reasons. Turner reported on ballistic gene-gun transfection of myeloma cells, but this method failed to produce high levels of transgene product (58). The main reason was the fragility of these cells (59). Systematic investigation of other nonviral gene transfer techniques such as electroporation, cationic polymers, or receptor-mediated transfer in myeloma cells is warranted to establish methods that avoid infectious agents. Up to now, no vector type can be propagated as an ideal vector to transfect myeloma cells.

Gene Transfer in Whole Myeloma Cell Vaccination. The rationale for genetically modified myeloma cell vaccines is to augment the immunogenicity of myeloma cells. Most of the various preclinical studies apply *ex vivo* transfection and subsequent readministration of irradiated myeloma cells as vaccine in animal models. Candidates for transfection are genes that encode cytokines (IL-2, IL-12, GM-CSF), costimulatory molecules (B7.1 or B7.2), and MHC molecules (Fig. 1). In the concept of cytokine-gene-transfected myeloma vaccines, it is important that cytokines be produced at high local concentrations near the myeloma cell. Thus, proinflammatory side effects of systemic cytokine administration can be avoided. Furthermore, the paracrine way of action much more closely resembles the natural cytokine physiology.

IL-2 is one of the most important antitumor cytokines because of its capabilities for clonal expansion of antigen-specific T cells and stimulation of nonspecific NK cells. IL-12 shows similar functions; this cytokine induces IFN-γ secretion by T and NK cells, enhances the proliferation of activated T cells and NK cells, augments the cytolytic activity of CTLs and NK cells, and supports the differentiation of Th1 helper effector cells (60). IL-12 therapy has been shown to reverse defects in NK cell and T cell function that are associated with advanced cancer in humans (61). Simultaneous transfection with genes encoding for the p35 and p40 chain is necessary for production of active IL-12. Expression of both the *IL-2* and the *IL-12* genes in several solid tumor models has been found to induce strong and specific antitumor immune responses. Interestingly, Wang *et al.* showed that IL-2 enhances the response of NK cells to IL-12 through up-regulation of the IL-12 receptor and signal transducer and activator of transcription protein 4 (62). These cytokines are particularly attractive for gene transfer strategies in MM. Lieu *et al.* (63) showed that the retroviral MSCVpac-mIL-12 vector directed robust expression of both the *p40* and the p*35* genes in a myeloma cell line. This vector may be a valuable tool in immune gene therapy of MM. Kopantzev *et al.* (64) transduced murine myeloma cell lines with a retroviral vector expressing the human *IL-2* gene. They observed that i.v. immunization of mice with irradiated, *IL-2* secreting cells led to significant protection from challenge with parental myeloma cells.

GM-CSF is another interesting transgene product in tumor cell vaccines because of abilities to promote maturation of precursor cells into DCs. The accumulation of these activated APCs in the microenvironment of tumor cells is expected to induce generation of tumor-specific T cells. Turner *et al.* (58) demonstrated high efficacy for a tumor cell vaccine consisting of a myeloma cell line (MPC11) and fibroblasts transfected with *IL-12* and *GM-CSF* genes using particle-mediated gene transfer in the poorly immunogenic murine myeloma MPC11 model. Injection of this vaccine induced tumor rejection in 60% of the mice, whereas control MPC11 provided no protection. Furthermore, this group confirmed this efficacy for a multidrug resistant isogenic subline of MPC11-cells, which suggested that P-glycoprotein-mediated multidrug resistance does not interfere with lysis by CTLs (65). Because selection of myeloma cells with multidrug resistance phenotype by chemotherapy is a frequent problem, this approach may be attractive in the treatment of chemotherapy-resistant MM. Li *et al.* (66) investigated vaccines consisting of a retroviral-transduced myeloma cell line (B9BM1) expressing single or combinations of transgenes (*GM-CSF*, *IL-12*, *CD80*, *Flt3L*) in a mouse model. They demonstrated that the combined use of *GM-CSF*, *IL-12*, and *CD80* is superior to the use of any single gene product in protection of s.c. challenge with parental tumor cells.

B7–1 (CD80) and B7–2 (CD86) play a critical costimulatory role in the activation of TCR-stimulated CTLs by binding to CD28. Blocking the interaction between B7 and CD28 can result in functional anergy to antigen stimulation (67). Transfection of the encoding genes of these molecules induced potent antitumor immune responses in a variety of tumor models (68). Myeloma cells often show little or no expression of B7 antigens (69). Tarte *et al.* (69) were able to demonstrate stimulation of allogeneic CD8+ T-cell proliferation after retroviral transduction of myeloma cells with the *B7–1* gene. For one patient with advanced disease, *B7–1* gene transfer made it possible to amplify autologous CTLs, which killed autologous myeloma cells

in a HLA class I-restricted manner. Similarly, Wendtner *et al.* (52) observed that gene transfer of *B7–1* and *B7–2* using adeno-associated viral vectors into human myeloma cells enhances cytolytic response and secretion of cytokines (IL-2 and IFN-γ) of human allogenic T cells. These results suggest that *B7–1* gene transfer is of great promise in immunotherapy of MM.

CD40 ligand (CD154) is a membrane glycoprotein, transiently expressed on T-helper cells, that binds to CD40 in APCs and, thus, induces expression of accessory costimulatory molecules. Adenovirally transfected autologous chronic lymphocytic leukemia cells, increased numbers of specific T cells, and reductions of leukemia cell counts and lymph node size have been demonstrated in a clinical study in 11 chronic lymphocytic leukemia patients treated with CD40 ligand (70). Bashey *et al.* (53) transfected myeloma cells with the gene for CD40 ligand using an adenoviral vector (Ad-CD154). High transfection efficiencies up to 98% could be achieved. Interestingly, myeloma cells were more sensitive for adenovirus infection than other cell types in the bone marrow aspirate. Transfected myeloma cells were shown to induce other costimulatory molecules (such as CD80, CD86 and CD54) on cocultured CD40-bearing B cells. A clinical study using this approach in myeloma patients would be of great interest. Dotti *et al.* (71) tested the hypothesis that expression of CD40 ligand in the region of a myeloma cell vaccine might trigger tumor specific immunity by recruitment of APCs. Mice were inoculated s.c. with two poorly immunogenic murine myeloma cell lines (MPC-11 and S107) mixed with irradiated CL7.1 fibroblasts that had been retrovirally transduced to express mCD40 ligand. For both cell lines, coinjection with mCD40 ligand and fibroblasts significantly slowed tumor cell growth. Thus, CD40 ligand transfection may play a role in additional strategies of immunotherapy in MM.

As in several other malignancies (72), in MM, genetically modified tumor cell vaccines resulted in protection from challenge by additional local injections of nontransfected parental tumor cells and, occasionally, in rejection of established tumors in animal models. However, it has always to be kept in mind that it is difficult to extrapolate such data from animal experiments to humans. For example, the systemic antitumor immune response to a cancer vaccine in humans may be less sufficient than in mice because of a higher complexity of the human immune system. Furthermore, the frequently used s.c. myeloma animal models (65, 66, 71) may not represent the sensitivity of orthotopic bone marrow myeloma cells. Nevertheless, the above reviewed studies in animal models provide a proof of principle that should be undertaken before transduction of such preclinical approaches to a clinical trial.

DNA Vaccines. Strategies using vaccination with tumor antigen encoding DNA are currently in development. Stevenson *et al.* (73) created plasmid-DNA vaccines encoding for scFv. Injected in mouse muscle, this vaccine showed only poor anti-Id humoral immune responses and poor tumor protection. However, after fusion of tumor-derived scFv-DNA to the fragment C sequence of tetanus toxin, the vaccine elicited protective immunity against tumor challenge in a mouse myeloma model. The fusion of Id sequences to a pathogen sequence may be advantageous in overcoming immune tolerance to tumor antigens. Clinical trials using this strategy in MM are planned (74). Furthermore, DCs are very attractive targets for transfection of TAA genes. Retroviral transduction of DC precursors of myeloma patients with scFv sequences is currently under investigation (74).

Antisense Therapy. Antisense oligonucleotides can inhibit expression of genes essential for the growth of malignant cells by binding to its complement mRNA. Various approaches of antisense therapy in MM are explored. Macrophage inflammatory protein 1-α has been recently identified as a factor produced by myeloma cells that may be responsible for the bone destruction in MM. Choi *et al.* (75) transfected the human MM cell line ARH-77 with an antisense construct to macrophage inflammatory protein 1-α (AS-ARH) and studied the capacity of these cells to cause bone disease in SCID mice. Mice treated with AS-ARH cells lived longer and had no lytic lesions compared with control mice receiving empty vector ARH cells, which had extensive bone disease. Survivin, which blocks apoptosis and is detected in most tumors, may be another interesting target in MM. Tamm *et al.* (76) showed that down-regulation of survivin in myeloma cells by antisense treatment leads to increased apoptosis and sensitivity to chemotherapeutic drugs. A third interesting antisense oligonucleotide, targeting *Bcl-2* and termed G3139, has been shown to down-regulate *Bcl-2* and to produce clinical responses in patients with drug-resistant B-cell lymphoma (77). Recently, van de Donk *et al.* (78) showed that *Bcl-2* RNA and Bcl-2 protein could be down-regulated effectively in myeloma cell lines resulting in enhanced cytotoxicity of doxorubicin. Therefore, G 3139 is a candidate for a clinical study in MM. However, it has to be mentioned that the low uptake of antisense molecules by target cells *in vivo* is a persistent problem, which hinders a broader clinical use of antisense therapies in hematological malignancies such as chronic myelogenous leukemia (79).

Gene Transfer of Suicide Genes in Donor Lymphocytes. GVHD is a main cause for morbidity in allogeneic transplantation. T-cell depletion of the transplants reduces the incidence and severity of GVHD but weakens the GVM effect. Reinfusion of donor CD4+ T lymphocytes at relapse after transplantation often induces remission, but GVHD occurs in the majority of these patients. Transduction of T lymphocytes with the suicide gene *HSV-TK*, allows the elimination of them specifically by the administration of ganciclovir in case GVHD develops. Transfected cells metabolize ganciclovir to a toxic triphosphate. To evaluate the feasibility of this approach, Munshi *et al.* (80) transduced anti-CD3-stimulated primary human lymphocytes, cultured in IL-2, very effectively by a retroviral vector that carried the *TK* gene. Furthermore, the authors showed that TK-transduced cells can be killed very effectively by exposure to clinically achievable concentrations of ganciclovir. On the basis of these data, a clinical study has been initiated in patients with persistent or relapsing MM after T-cell-depleted allo-SCT. Unfortunately, selective *in vivo* gene transfer of a suicide gene like HSV-TK in myeloma cells is currently impossible. However, it is hoped that the development of more selective vectors will enable the applicability of suicide gene therapy in direct antimyeloma approaches.

Additional Approaches. Donovan *et al.* (19) reported on the generation of an anti-CD38 scFv construct that acts as the carrier of a toxin gene instead of being conjugated directly to the toxin itself. It is hoped that expression of the toxin by CD38+

plasma cells will promote suicide of the malignant cells without affecting normal cells or generating an immunological response to the toxin. Saggio *et al.* (81) constructed a recombinant adenovirus expressing the IL-6 receptor superantagonist, which blocked the IL-6-dependent proliferation of human myeloma cells and which, therefore, offers a means of long-term blockade of IL-6 activity *in vivo*.

Clinical Studies of Gene Therapy in MM Compared with Other Cancers. The clinical evaluation of gene therapy in MM is less advanced compared with several other malignant diseases. The majority of clinical trials have been performed in melanoma (82); genetically modified whole-cell vaccines have also been investigated in clinical trials in neuroblastoma, lung cancer, pancreatic cancer, prostate cancer, sarcoma, ovarian cancer, colon cancer, and lymphoma (72, 83). The relatively small number of patients preclude statistically significant evaluation of efficacy at this time. However, several complete, partial, and mixed responses as well as disease stabilizations were reported in these studies. For example, in our trial applying simultaneous nonviral transfection of *IL-7* and *GM-CSF* genes in 10 patients with various metastatic solid tumors, one complete, one partial, and one mixed response were observed. Two patients showed stable disease and five patients remained in progressive disease (83). These encouraging results suggest investigation of genetically modified whole cell vaccines in larger randomized Phase III studies.

To our knowledge only one group has published results of clinical application of gene modified myeloma cell vaccination. Stewart *et al.* (84) tested adenoviral vectors expressing IL-2 (AdCAIL-2) in two Phase I studies. First, these investigators injected the AdCAIL-2-vector directly into tumors of two patients with multiple s.c. plasmacytomas. The vector and IL-2 expression could be detected in tumor biopsies. Subsequently, the authors initiated an additional Phase I trial to evaluate s.c. vaccination with AdCAIL-2-engineered autologous plasma cells in patients with MRD posttransplant. Preliminary results indicate that this approach is feasible and is associated with only mild toxicity (local inflammatory response and occasionally flu-like syndrome). The clinical benefit cannot be valued yet (85).

The development of other gene therapy strategies in MM, such as DNA vaccination or antisense therapy, is still at the level of preclinical evaluation. In other malignant diseases such as colon carcinoma (86), melanoma, and lymphoma, clinical DNA vaccine studies are ongoing (87). Antisense gene therapy is currently being investigated in clinical Phase I/II trials in lymphoma, acute and chronic myelogenous leukemia, and in a variety of solid tumors (ovarian, prostate, breast, pancreas, brain, colon, and lung; Ref. 88).

Munshi *et al.* (80) reported on the initiation of a clinical trial applying *HSV-TK* suicide gene-transduced lymphocytes in patients with persistent or relapsing MM after T-cell-depleted allo-SCT. However, clinical response data are not yet available. In a previous clinical study, GVHD in three lymphoma patients could be effectively controlled by ganciclovir-induced elimination of the transduced donor lymphocytes (89).

Gene Marking in Autologous PBSCT. Gene marking has no direct therapeutic effect but may provide valuable information to improve treatment based on autologous SCT. To answer the question whether relapses of MM after autologous PBSCT are attributable to persistence of myeloma cells surviving high-dose chemotherapy or are attributable to contamination of the graft with myeloma cells, Gahrton *et al.* (90) conducted a gene-marking study using a retroviral vector (G1Na). CD34-positive cells from eight patients were incubated with the G1Na vector encoding the bacterial *NeoR* marker gene. Transduction efficiencies ranged from 0.5 to 5.1%. These investigators showed that it is possible to identify the marker gene *in vivo* after transplantation. In a recent follow-up analysis in one of three relapsed patients who were analyzed, the marker gene has not been identified in myeloma cells (91). Further follow-up is needed to assess the utility of this approach in MM.

Prospects of Multimodality Treatments

Myeloablative chemotherapy is currently the most effective therapy for MM. However, long-term efficacy remains unsatisfactory and further increase of intensity is impossible because of intolerable toxicity. Most immunology-based therapies have a very low toxicity and can be, therefore, administered to the patient in addition to cytostatic drugs. Furthermore, because they are non-cross-reactive to chemotherapy, synergistic effects can be expected. The combination of conventional treatments with immunotherapy and gene therapy or other molecular strategies may be the way to eradicate, or at least control, MRD. It is generally believed that the tumor burden should be minimized by chemotherapy prior to immunotherapy to obtain optimal results (74). However, whether the immune system, especially the T-cell compartment of patients after intensive chemotherapy, is competent enough to elicit clinically relevant antitumor immune responses has to be monitored carefully in clinical studies. The full potential of biological therapy may finally be realized by combining different approaches to immunological therapy, gene therapy, and other molecular strategies that show synergistic antimyeloma effects (*e.g.*, rituximab and IFN) without an increase in severe toxicity.

Conclusion

Because of the limited efficacy of conventional therapies, new strategies in the treatment of MM are required. Numerous approaches of biological therapy are currently under investigation. Induction of specific antimyeloma immune responses has been demonstrated in a multitude of studies and confirmed in animal models. The first clinical trials are at early stages and results are very preliminary. However, gene therapy as well as immunotherapy in MM is only beginning to realize its potential because many technical obstacles still need to be overcome. Considering a continued progress in optimization of gene transfer techniques and understanding of tumor immunology molecular therapies are extraordinarily promising for the future in the treatment of MM.

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